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Effects of ocean acidification on dispersal behavior in the larval stage of the Dungeness crab and the Pacific Green Shore crab

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**EFFECTS OF OCEAN ACIDIFICATION ON DISPERSAL BEHAVIOR
IN THE LARVAL STAGE OF THE DUNGENESS CRAB AND
THE PACIFIC GREEN SHORE CRAB**

By

Anna-Mai Florentine Christmas

Accepted in Partial Completion
of the Requirements for the Degree
Master of Science

Kathleen L. Kitto, Dean of the Graduate School

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MASTER'S THESIS

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Anna-Mai F. Christmas

October 15, 2013

**EFFECTS OF OCEAN ACIDIFICATION ON DISPERSAL BEHAVIOR
IN THE LARVAL STAGE OF THE DUNGENESS CRAB AND
THE PACIFIC GREEN SHORE CRAB**

A Thesis

Presented to

The Faculty of

Western Washington University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science

By

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October 2013

ABSTRACT

The influence of acidification of the world's oceans on marine populations and communities is a subject of growing concern. In the case of crustaceans, issues such as calcium dynamics of the molting process and direct effects on survival and development rates of larvae have received, at most, limited attention. My thesis research looked at phenomena that are important in the success of larval crustacean stages, but have received no attention; namely, the effects of ocean acidification on the swimming speeds, feeding rate, and gross growth efficiency of stage one larvae of the Dungeness crab, *Metacarcinus (Cancer) magister*, and the Pacific Green Shore crab, *Hemigrapsus oregonensis*.

For five days, the larvae of these crab species were held in carbon dioxide enriched seawater at the current atmospheric value for the control treatment (400 ppmv) and the projected level for the year 2100 (IPCC) for the high treatment (1000 ppmv). After Day 1 and Day 5, swimming behavior of the larvae was tested by looking at their distance travelled over time (orthokinesis) and the number of turns taken over time (klinokinesis). Their feeding rates were also compared by measuring the number of *Artemia* sp. nauplii consumed and gross growth efficiency was tested by measuring larval growth in calories divided by the total number of calories consumed.

There was no significant difference found in swimming behavior, feeding rates or gross growth efficiency of either *M. magister* or *H. oregonensis* larvae between the CO₂ treatments on each day. In both species, the swimming behavior and number of turns were higher on Day 1 when compared to Day 5. Results for the feeding rate showed an increase after five days for both species. However, after five days of exposure to acidified seawater, *M. magister* larvae had a significantly higher rate of turning than did those placed in the control CO₂ conditions. This

shows that high levels of ocean acidification may not directly affect feeding rates, the efficiency with which larval *M. magister* and *H. oregonensis* transform energy consumed into growth or the total distances travelled per unit time,. But, it may have an effect on larval movement patterns which may in turn affect larval vertical distribution and dispersal.

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Introduction

Crustaceans make up 30% of the fisheries revenue in the United States (Cooley, 2010). In Washington State, over \$73 million is generated per year from crustacean fisheries, supporting approximately 2,000 jobs (Washington Department of Fish and Wildlife, 2013). Commonly captured species include the spot prawn, *Pandalus platyceros*, the coonstripe shrimps, *P. danae* and *P. hypsinotus*, the Pink shrimps *P. eous* and *P. jordani*, the red rock crab, *Cancer productus*, and the Dungeness crab, *Metacarcinus magister*. According to the Washington Department of Fish and Wildlife (2013), 8.75 million pounds of *M. magister* were harvested recreationally from the Puget Sound in the 2010-2011 season alone. Because crabs play such a major role in the fishing industry, it is important to understand the factors that can influence their adult population abundance and distribution, including events that occur during their larval stages.

Crab species have a life history that includes a free-swimming larval stage, the zoea. Crab larvae molt periodically as they develop, with consecutive zoeal stages increasing in size and morphological complexity and developing new physiological and behavioral traits (Pechenik, 1999). After a set number (per species) of zoeal stages, the crabs molt into an intermediate post-larval form, the megalopa, that transitions from a pelagic to primarily benthic habit, seeks out suitable settlement sites for juvenile life and undergoes metamorphosis. All larval stages are planktotrophic; that is, they must feed in the plankton to survive.

At some times and in some places, crab zoeae can dominate the mesozooplankton (Sulkin, unpublished). Zoeae are important prey for jellyfish, finfish, and other zooplankters and are, themselves, predators on a wide variety of other planktonic organisms (Bigford, 1978; Harms & Seeger, 1989; Paul et al., 1989; Lehto et al., 1998; Sulkin et al., 1998; Perez & Sulkin,

2004). Accordingly, their abundance and distribution can have population consequences to the crab species themselves and consequences to the broader pelagic community by influencing predator-prey interactions via both top-down and bottom-up processes. Localized abundance of the zoea, as well as distribution and abundance on a larger scale can be controlled by a variety of factors including differential dispersal and mortality due to water quality, predation, and nutritional stress (Sulkin et al., 1998; Sulkin & McKeen, 1989).

Feeding behavior in larval crabs is a complex process that involves initial capture, manipulation of ingestion and digestion/assimilation. The larvae are opportunistic encounter feeders, basically capturing and attempting to ingest whatever particles they “bump into” (Hinz et al., 2001). They are omnivorous, ingesting microalgae, micro- and meso-zooplankton, and even detrital particles ranging from a few microns in diameter (microalgae) to 450 μ in length (brine shrimp nauplii). Although they are omnivorous, they must include mesozooplankton in their diets to develop normally. Although they ingest a wide variety of particles, they are selective when exposed to some species of toxic algae (Hinz et al., 2001; Perez & Sulkin, 2004). The efficiency with which crab larvae assimilate ingested prey has been studied by Mootz & Epifanio (1974) and Levine & Sulkin (1979), but the effects of water quality on the process have not been investigated.

The swimming activity of crab larvae can lead to changes in their depth and position of larval crabs in large water masses. Such changes can, in turn, affect the dispersal of the larvae. Dispersal of larval crabs is influenced by a variety of morphological, environmental and behavioral factors (Sulkin et al., 1983; Sulkin, 1984; Forward, 1985; Gherardi, 1995). These factors interact to regulate depth in the water column. In a stratified coastal system, depth can greatly influence dispersal. Retention of larvae in suitable habitats and recruitment to good

locations are determined by the interaction of internally and externally controlled behaviors and hydrographic features (Sulkin, 1984; Little & Epifanio, 1991; Olmi, 1994).

Water quality can have both direct (mortality, growth rate) and indirect (feeding encounter rate, swimming behavior) effects on larval distribution, survival and growth. Features including salinity, temperature, and toxicants can directly affect behavior or survival (e.g., Sandoz & Rogers, 1944; Costlow & Bookhout, 1959; Sulkin, 1984; Sulkin & McKeen, 1989). One water quality feature that could influence larval crab development and behavior is currently receiving a great deal of attention: ocean acidification. Acidification of ocean waters, a result of increasing atmospheric CO₂ levels, is a process that has been occurring since the industrial revolution and appears to be accelerating in recent decades (Feely et al., 2009). It has been predicted that ocean pH will decrease by 0.4-0.6 units by the end of this century (Caldeira & Wickett, 2003). Increased acidification of sea water involving interactions among CO₂, carbonic acid (H₂CO₃), bicarbonate (HCO₃⁻), and carbonate (CO₃²⁻), has a variety of consequences to marine chemistry. The relative concentrations of these moieties depends upon pH (Archer, 2007). Among the consequences of changes in abundance of these compounds are changes in the dynamics of calcium, a principal element of internal and external skeletons in many marine organisms, including marine arthropods. The effects of these acidification-related changes in marine chemistry have been the subject of considerable recent study (e.g., Ries et al., 2009). The potential impacts on marine phytoplankton and changes in system productivity that could ensue have received particular attention (Riebesell et al., 2000; Riebesell, 2004).

With the potential effect of acidification on shell-bearing invertebrates, direct research of acidification on the larval stage of crustaceans has been greatly discussed. Arnold et al. (2009) reported a reduction in carapace mass of the final larval stage of the European lobster *Homarus*

gammarus due to a reduction in exoskeletal minerals (calcium and magnesium). However, effects of exposure to sea water that has been acidified to the level projected for the end of this century on behavior of any larval crustacean has not yet been determined. Acidification could have direct and indirect impacts on behavior and energetics. For example, changes in the intrinsic activity levels of larval crabs could have significant consequences both to the behaviors that determine depth regulation (and hence, dispersal) and to energetics.

The objective of my research was to determine whether changes in the acidity of ocean waters impact crab larval swimming, feeding and growth efficiency. Does exposure to acidified sea water change the intrinsic swimming activity of larval crabs? Do changes in the ocean's pH affect their feeding rates on zooplankton prey? Will exposure to acidified sea water change the gross growth efficiency? These studies were conducted on the first zoeal stage of two crab species, the commercially important and winter spawning species, *Metacarcinus magister* (Dungeness crab), and the summer spawning species, *Hemigrapsus oregonensis* (Green Shore crab).

METHODS

Experimental Approach

To determine the effects of ocean acidification on larval crab behavior and physiology, swimming activity, feeding rates, and gross growth efficiencies were compared in larvae exposed to seawater at pH levels that typify current ambient atmospheric CO₂ levels (control) and to the high ocean acidification level predicted for the year 2100 (high). Swimming activity was measured both as the distance travelled per unit time (orthokinesis) as well as the total number of turns per unit time (klinokinesis). Feeding rates were measured by determining the number of *Artemia* sp. nauplii consumed by larvae in the treatments per unit time. Gross growth efficiency was determined by dividing the growth in calories over a specified time interval (the change in dry weight of the larvae over time X the calories per unit dry weight) by the total calories consumed (the number of prey consumed per day X the calories per prey organism X the number of days of feeding).

Experimental Organisms

Ovigerous Females and Crab Larvae

Metacarcinus (Cancer) magister (Family Cancridae) was chosen as an experimental species because of its larval availability between mid-January and late March. *Hemigrapsus oregonensis* (Family Grapsidae) was used because of its summer availability and its abundance in the local area. These two species provided a comparison between two families of brachyuran crabs and between winter and summer spawning species.

Metacarcinus magister egg-bearing females, ranging from 15-18 cm in carapace width, were collected on February 6, 2012 from Ship Harbor, Anacortes, Washington, USA (Figure 1) by SCUBA divers. The crabs were kept in 0.5 X 0.5 x 0.32 m Plexiglas tanks with continuous seawater at 10.9-11.5°C and 30-33 psu until hatching began. When a female's eggs began to hatch, the tank was drained, cleaned of all zoeae, refilled and left overnight to ensure that all larvae collected for use in an experiment the next morning were less than 24 hours old. These larvae were designated as Day 1 larvae. Crab larvae were collected using a 253-µm screened filter and placed in the appropriate experimental set up.

Hemigrapsus oregonensis ovigers, ranging from 1.5-4 cm in carapace width, were collected on June 27, 2012 from the Shannon Point Marine Center beach, Anacortes, Washington, USA (Fig. 1). Females were kept in continuous flow containers at 10.9-11.5°C and 30-33 psu until heartbeat was observed in the brooded eggs. Once hatching or larval heartbeat was observed, a female was moved into a 20-cm diameter glass bowl filled with 0.2-µm FSW (30 psu) and placed in an incubator at 15°C with a 12-hour light-dark cycle. The water in the bowls was changed every other day until the brood hatched. The ovigers were checked daily for hatching and once hatching occurred, the larvae were removed and used in experiments within 24 hours.

Algal Cultures

Isochrysis galbana Parke (Strain 1323), a non-toxic alga purchased from Provasoli-Guillard National Center for the Culture of Marine Phytoplankton (CCMP) (Booth Bay Harbor, Maine, USA), was cultured in F/2 media. The cultures were kept aerated and in 24-hour light in a light box at room temperature (64-70°C). *I. galbana* has been used previously to culture rotifers



Figure 1: Map of collection sites for female crab ovigers. A: *Metacarcinus magister* ovigers. B: *Hemigrapsus oregonensis* ovigers.

for everyday larval crab feeding (Sulkin & McKeen, 1989) and were used to create cultures of rotifers to use as a maintenance food for the larval crabs used in the study.

To create a mass *I. galbana* feeding culture, the starter culture obtained from CCMP was incubated at 15°C with a 12 hour light – dark cycle. The F/2 recipe used for the culture medium was obtained from CCMP and consisted of a 1:1:1:0.5 ratio of nitrate, phosphate, trace metals and vitamins, respectively. A 20-L glass Kimble carboy was filled with 0.2-µm filtered sea water (FSW) that had been pasteurized by heating it to 70°C for 30 minutes while being aerated to ensure that heat was evenly circulated throughout the seawater. Once this seawater cooled to room temperature (approximately 24 hours), F/2 nutrients and an *I. galbana* starter culture were added. The carboy was then placed in a 24-hour light box at room temperature and was bubbled constantly using an aquarium pump. To prevent overgrowth and crashing of the *I. galbana* cultures, they were diluted periodically to keep the cell densities at $1-2 \times 10^6$ cells mL⁻¹. Algal cells were harvested as needed to sustain mass cultures of the rotifer *Brachionus plicatilis* Muller that were in turn used to sustain the larval crabs.

Rotifer Cultures

Cultures of the rotifer *Brachionus plicatilis* were obtained from Reed Mariculture, Incorporated (Campbell, California, USA). Rotifers were filtered out of algal medium by passing the culture through an 80-µm filter and rinsing with 0.2-µm FSW. Rotifers were maintained in 500 ml glass beakers filled with *Isochrysis galbana* until needed for everyday larval feeding. These beakers were labeled with the algal name and feeding dates to maintain the rotifer feeding schedule. The beakers were then covered with a clean plastic petri dish to prevent contamination, and kept in a 20°C incubator on a 12-hour light – dark cycle. To maintain algal densities

adequate to keep the rotifer cultures in an asexual reproductive phase, the rotifers were fed twice a week by sieving them out of the old *I. galbana* culture through an 80- μ m filter and re-suspending in a clean 500-ml glass beakers filled with new *I. galbana*.

***Artemia* sp. Cultures**

Artemia sp. cysts were obtained from Argent Chemical Laboratories (Redmond, Washington, USA) and were stored in plastic containers in a 15°C incubator. *Artemia* sp. nauplii were hatched and harvested as needed for crab feeding rate experiments. For hatching to occur on the initial day of each experiment, the cysts were prepared 24 hours before an experiment was started. A 2-L plastic *Artemia* sp. hatchery stand was filled with 1.5 L of 0.2- μ m FSW and half a teaspoon of *Artemia* sp. cysts. The stand was covered and placed under a high intensity light for 24 hours. An aquarium pump was used to aerate the water and increase circulation. Another batch of *Artemia* sp. cysts was harvested on day four to be used in the Day 5 feeding rate experiments.

Ocean Acidification System and Measurements

The ocean acidification system employs air compressors to pump ambient air from outside through a filter then through a mass flow controller that controls the amount of CO₂ in the air by mixing pure CO₂ gas with the filtered air in specified proportions. This CO₂-enriched air is separated into two areas, one containing of 15-L carboys for conditioning seawater and another consisting of sealed experimental chambers (Fig. 2). The 15-L carboys were filled with autoclaved 0.2- μ m FSW kept at 15°C in a dark incubator to prevent algal growth. Air is forced into the sea water-filled carboys where equilibration to the desired CO₂ level is achieved by vigorous bubbling. The second area consists of sealed experimental chambers containing the

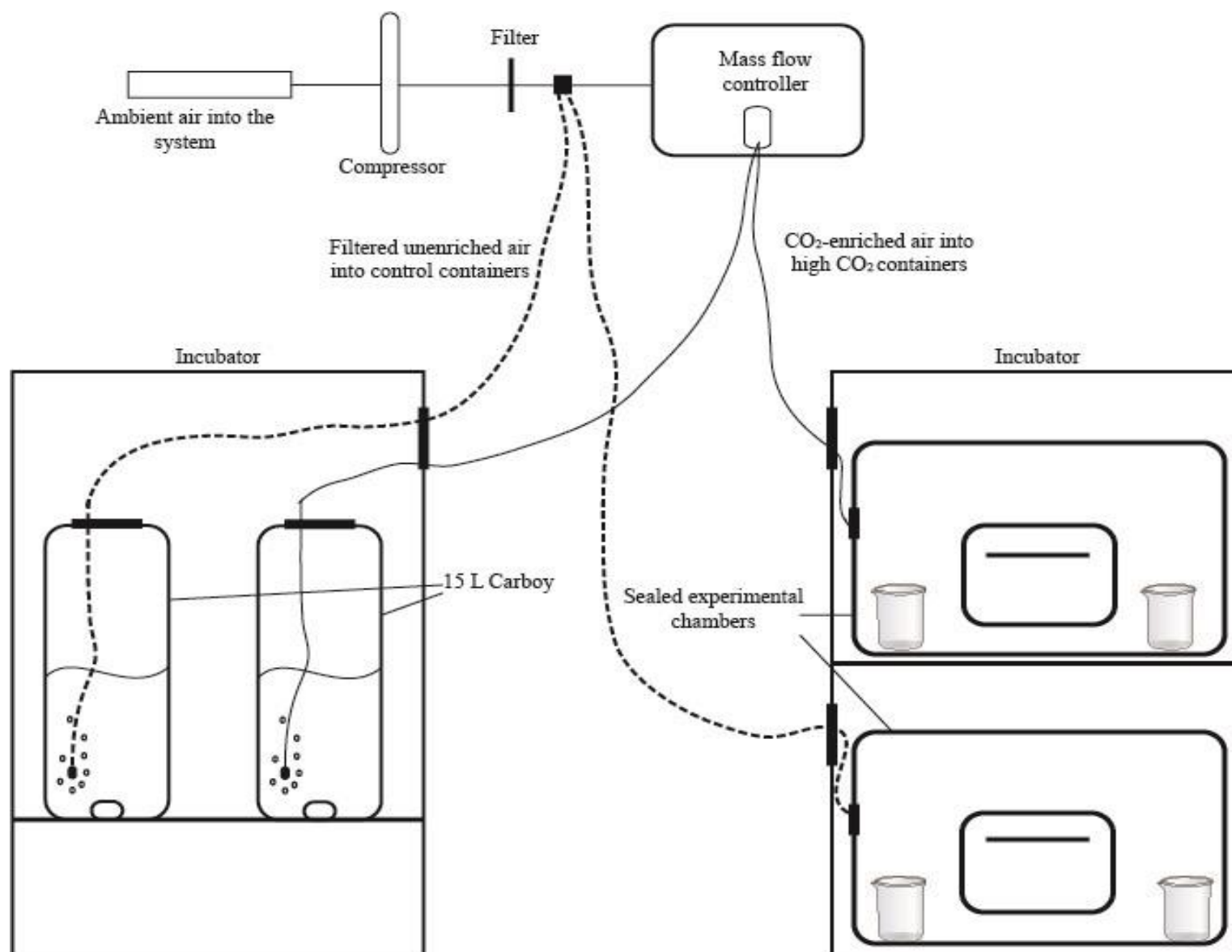


Figure 2: Diagram of ocean acidification system. See text for details.

beakers in which experiments with larvae were conducted. These chambers simulated the air-to-water CO₂ exchange characteristic of a high CO₂ environment. The experimental chambers in this area were kept in a 15 °C incubator with a 12-hour light-dark cycle. A parallel control system was set up similarly with the exception that pure ambient outside air was passed through a filter then sent directly into the two areas without CO₂-supplementation. The final CO₂ for this non-enriched control condition measured between 400-420 ppmv in micro-atmospheres and the CO₂-enriched system measured 1000 ppmv in micro-atmospheres.

To monitor pH daily, 30-mL of seawater from the control and high CO₂ treatments were placed in 10-cm cuvettes. These samples were taken during the daily water changes. The cuvettes were placed in a temperature controlled chamber for one hour to bring their temperature to 25°C. pH was determined using a spectrophotometric method with the indicator dye m-cresol purple and a diode array spectrophotometer (Easley & Byrne, 2012; Rerolle et al., 2012). Salinity of each sample was measured in psu using a refractometer.

Subsamples of both the control and high CO₂ seawater treatments were also taken for alkalinity measurements on days 0, 1, 3, and 5. A 100-mL sample from each treatment was filtered through a 0.7-μm pore size 25-mm diameter glass fiber filter, placed in a 100-mL glass bottle, preserved with 0.2-μL mercuric chloride and stored in a 6 °C dark incubator. For days 1, 3, and 5, the subsamples were taken during the daily water changes. Day 0 samples were taken directly from the carboys to determine the initial alkalinity. Alkalinity was analyzed using a Metrohm 888 Titrando titrator with a Metrohm Ecotrode combined electrode using 0.1 N hydrochloric acid and sodium chloride; corrected with QC solution, a certified reference material (Dickson et. al., 2007). Salinity of each sample was measured using a refractometer. Using measured salinity, temperature, pH and alkalinity, *p*CO₂ was determined for each sample with

the CO2SYS program using the Dickson and Millero (1987) constants of Lewis & Wallace (1998; Table 1).

Preliminary studies were conducted to determine the number of larvae and rotifers that could be placed into the various ocean acidification treatments without significantly changing the pH and $p\text{CO}_2$ levels due to respiration. It was determined that no more than 80 *Metacarcinus magister* larvae or 100 *H. oregonensis* larvae in 400 mL of water and five rotifers per larva could be held in the containers without moving the pH from its nominal levels (7.5 for high pH and 7.8 for control).

Larval Exposure

The *Metacarcinus magister* experiment began when two broods hatched on the same day. The Plexiglas tanks in which the ovigerous crabs had been held were emptied and refilled once hatching was observed. Within 24 hours, newly-hatched larvae were carefully removed from the water by sieving them through a 253- μm screened filter. Larvae from both broods were placed into a glass bowl and mixed together to account for possible brood effect. Fifty larvae were placed in beakers of 400 mL of control or CO_2 -enriched seawater; eight beakers per treatment. These beakers were then placed in their appropriate experimental chambers in the ocean acidification system.

When two broods of *Hemigrapsus oregonensis* hatched on the same day, larvae from both broods were collected by sieving them out with a 100- μm filter and mixing them together to account for possible brood effect. Eighty larvae were placed in 10 beakers of 400 mL of control or CO_2 -enriched seawater; five beakers per treatment. These beakers were then placed in the ocean acidification experimental chambers. For each experiment, a subsample of these larvae

Table 1: Parameter values in control and CO₂-enriched seawater obtained within the ocean acidification system during the five-day experiments

Parameter	Control	CO ₂ -Enriched
Salinity	31.51 ± 1.52	31.51 ± 1.52
Temperature (°C)	15.93 ± 0.16	15.92 ± 0.15
pH	7.80 ± 0.04	7.53 ± 0.03
Total Alkalinity (µequiv kg ⁻¹)	2116.09 ± 68.69	2162.62 ± 121.49
<i>p</i> CO ₂ (µatm)	506.29 ± 60.30	1031.16 ± 79.09

was removed from each beaker. Ideally, each larva would have been treated independently in its own container instead of being treated in batches. This batch treatment could be considered pseudoreplication (Hurlburt, 1984). This approach was unavoidable due to the limitation of the ocean acidification system and the results need to be interpreted recognizing this feature of the design.

The seawater in the beakers was changed every day to minimize the chances of elevated $p\text{CO}_2$ values from larval respiration. This was accomplished within a one-hour interval as previous work has shown that when the beakers are removed from the experimental chambers, the pH level can be maintained for only short periods (Garcia, unpublished). Water changes were done by removing the beakers from the experimental chambers, sieving the larvae through a 100- μm filter and gently rinsing with the appropriately treated seawater. A new clean labeled beaker was filled with 400 mL of either control or high $p\text{CO}_2$ seawater and the larvae were returned to their treatment. From the used water in each treatment, two 30-mL subsamples were taken every day for pH measurements and one 100-mL subsample was taken on experimental days 1, 3, and 5 to obtain alkalinity values.

The crab larvae in the beakers in the experimental chambers were fed rotifers daily. Rotifers cultured on *Isochrysis galbana* are good for larval crab feeding as they have effectively sustaining larval growth (Sulkin & McKeen, 1989; Garcia et al., 2011). Each day during the experiment, rotifers maintained on *I. galbana* in the batch cultures used for feeding were filtered through an 80- μm sieve and rinsed with 0.2- μm FSW to remove any *I. galbana* residue. The rotifers were then re-suspended in a beaker of 100 ml of 0.2- μm FSW. A 5 mL aliquot was taken and fixed with 1 mL 90% ethanol in a 20-mL scintillation vial and a Sedgwick Rafter counting cell was used to determine rotifer density. The density obtained was then used to determine the

volume of the batch culture needed to feed the crab larvae a density of five rotifers per larva. The specified density of rotifers was fed to the crab larvae every day when their water was changed. The crab larvae that had been fed these rotifers were then used in the swimming speed, feeding rate, and gross growth efficiency experiments.

Swimming Behavior Study

This study involved measurements of larval swimming behavior after the larvae had been kept in high and control levels of CO₂ in the ocean acidification system. Swimming measurements were made after the larvae had been kept under experimental conditions for 24 hours and again after five days of exposure. Each day, the larvae were moved into new beakers of 400 mL conditioned water and were fed *Isochrysis galbana*-fed rotifers. The *Metacarcinus magister* experiment began when two broods hatched on March 14, 2012 and the *Hemigrapsus oregonensis* experiments began on July 18, 2012. After 24 hours of exposure, 30 larvae were removed from each treatment (five larvae from each of six beakers) and used in Day 1 swimming speed experiments. The experiment was repeated after five days of conditioning to assess longer-term acidification effects.

Swimming Speed Experimental Setup

A Sony HD digital camera was used to record larval swimming (Fig. 3). The camera was attached to a camera stand 20 cm over a clear Plexiglas stand holding a 3.5-cm diameter petri dish. A red light (~650 nm) was placed under the platform pointing upwards towards the camera and all other lights were turned off. This was done to provide a single light source during the experiment at a wavelength the crab larvae did not respond to (Goldsmith and Fernandez, 1968; Sulkin, 1984). For each trial, a larva was placed in the plastic petri dish with 4 mL of conditioned

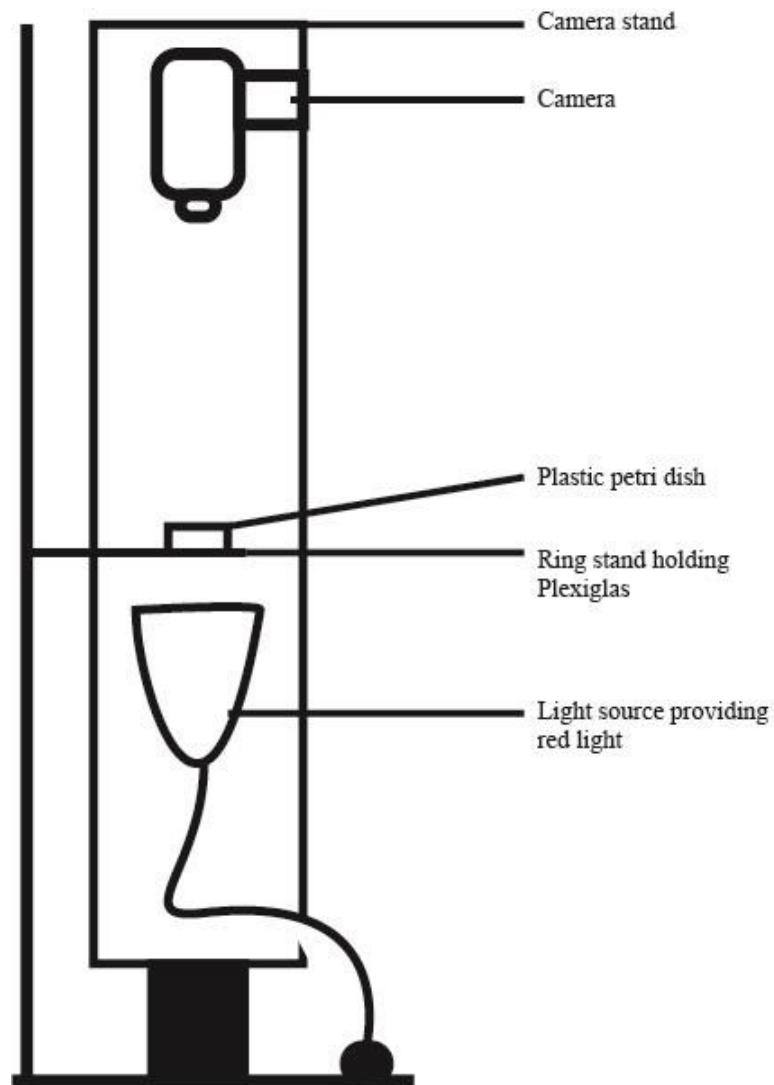


Figure 3: Experimental setup for measuring the swimming speeds for larval *Metacarcinus magister* and *Hemigrapsus oregonensis*. Larvae were placed in the plastic petri dish and swimming was recorded for two minutes.

water and its movement was recorded for two minutes.

Thirty individual larval swimming videos for each treatment per day were made then DVDVideoSoft software was used to capture images from the videos, taking 3 images every second for the full 2 minutes of the video. The Bioscan Optimas image analysis program was used to measure orthokinesis which was obtained by looking at the total distance individual larvae travelled over the two minute trial. This permitted calculations of the mean swimming speeds. The number of turns taken was used to determine klinokinesis, considering every angular movement over 10° between sequential images as a turn. Swimming speed and turns over time was analyzed both between the Days 1 and 5 and between the treatments on each day.

Feeding Rate Study

The feeding rates of larval crabs under control and high CO₂ in an ocean acidification system was determined by looking at the number of *Artemia* sp. nauplii consumed by newly hatched larvae over a 24-hour period. The feeding rate was again measured after five days of exposure to the treatments to evaluate the longer-term effects. Two hundred and twenty larvae each from *Metacarcinus magister* and *Hemigrapsus oregonensis* were used in this study. *Artemia* sp. nauplii was used in the feeding rate experiments instead of *Brachionus plicatilis* because they are easier to observe and count.

The experiment began when two or more crab broods hatched on the same day (March 14, 2012 for *M. magister* and July 18, 2012 for *H. oregonensis*). Larvae filtered from both broods were mixed together. Plastic 12-well trays were filled with conditioned water; five trays (60 wells) for high pCO₂ and five trays for controls. Eleven wells in each tray were filled with 5

mL of the appropriate water. One crab larva was arbitrarily selected and placed in each of the wells; providing a total of 55 larvae per treatment. Newly-hatched *Artemia* sp. nauplii were filtered from mass cultures using a 100- μ m sieve and placed in 0.2- μ m FSW. Ten of these nauplii were placed in each of the wells with the larval crabs. The trays were then placed in their appropriate experimental chambers within the ocean acidification incubation system. After 24 hours, the number of *Artemia* sp. nauplii consumed per well was determined by counting the number of *Artemia* sp. nauplii left in each well and subtracting it from the total initially placed in the well. Four days later, 55 larvae from the same batches that had been continuously held in treated conditions in the ocean acidification system were removed from the beakers in each treatment. These were placed in new, clean 12-well trays filled with treated water and the feeding experiment was repeated. The effects of the treatments on feeding rates were calculated by determining the number of nauplii consumed per unit time by larvae in each of the two treatments. The feeding rates in Day 1 high and control condition and the high and control values for Day 5 were compared.

Gross Growth Efficiency Study

Gross growth efficiency is a measure of an organism's capacity to transform energy consumed in food into energy in body tissue. This was determined for the crab larvae by dividing the growth in calories of a larva over a specified time period by the calories consumed during that same period. Data used in the calculation included the feeding rate of larvae on rotifers multiplied by the number of calories per rotifer multiplied by the number of days the larval crabs were fed. Data for the caloric content of rotifers were obtained from literature reports. Calories per unit dry weight of rotifers used in my study (5.05 ± 0.02 calories mg^{-1}) was reported by Theilacker and McMaster (1971) and Theilacker and Kimball (1984). The feeding

rate of *Metacarcinus magister* larvae consuming rotifers was reported to average 0.104 ± 4.82 mg of dry weight of rotifers larva⁻¹ day⁻¹ (Yúfera & Pascual, 1989; Burgess, 2011). This provided the total number of calories consumed. Growth of the larva over time in calories was obtained by multiplying the change in dry weight of the larva times the calories per unit dry weight of the larva. Calories per unit dry weight of the larva was determined using a Philipson microbomb calorimeter (Paine, 1971). The microbomb calorimeter is capable of detecting heat given off when very small samples are combusted in the bomb. The change in temperature is transformed into voltage, which is amplified by a DATAQ Analog Channel box. Those measurements are then interpreted and transformed into calories using WinDaq/XS Software and a calibration with benzoic acid, a substance of known caloric value. *Hemigrapsus oregonensis* larvae were not used in this experiment because there was no research done to determine the feeding rate of *H. oregonensis* on rotifer, which is needed to calculate the calories consumed.

Growth in Calories

When two crabs hatched on the same day, 15 larvae were haphazardly picked from the pool and placed in RO treated water for 15 minutes to remove salts to determine initial dry weight at hatching. These 15 larvae were placed together on a single pre-weighed Plexiglas plate and dried in a drying oven for 3 hours at 70°C. The plate was moved to a desiccator then weighed 48 hours later to determine the initial dry weight at hatching of a group of 15 larvae. After 24 hours and again after five days, 30 additional larvae were removed from each CO₂ treatment, separated into 2 groups of 15 larvae each, placed on pre-weighed Plexiglas plates and weighed as above. The difference in dry weight between the Day 1 group of larvae and the Day 5 group of larvae provided values for growth over time. All of the dried larval crab samples were

returned to the desiccator until they could be processed to determine the calories per unit dry weight.

Before any sample could be processed to determine the caloric content, the microbomb calorimeter had to be calibrated using benzoic acid. The benzoic acid was pelleted in a press then weighed to the nearest 0.01 mg. To prepare the microbomb, 3 inches of nichrome wire was inserted into the diode at one end and wrapped firmly around the other diode inside the bomb. A 90° bend was made in the center of the wire above the microbomb's plate. The benzoic acid pellet was placed on a 0.7 cm diameter circular platinum plate and the platinum plate was set onto the bomb plate directly under the 90° bent nichrome wire, ensuring that the bend was the only part of the wire touching the pellet. This caused the voltage to contact only one point on the pellet. The microbomb was closed tightly to ensure the pellet stayed in place under the bend, and the microbomb was pressurized to 420 psi with pure oxygen. Because the pressurization produced heat, the microbomb was cooled to room temperature in a glass bowl filled with deionized water. The microbomb was then dried and placed on the thermocouples, with the wires from the calorimeter connected to the microbomb contacts. The thermocouples detect the temperature changes in the bomb. The calorimeter was then covered with a Styrofoam box to prevent any ambient temperature changes from being registered by the thermocouples. The calorimeter was fired and the heat released from combustion of the pellet was recorded for 30 minutes using WinDaq/XS software.

Once the calorimeter had been calibrated, caloric content of the larvae was determined by powdering the groups of 15 dried larvae with a ceramic mortar and pestle. These crushed larvae were pelleted and processed as described above. Measurements from 2 groups of 15 larvae were obtained for each treatment for Day 1 and Day 5 larvae.

Using the equations in the bomb calorimetry manual by the Gentry Instruments Inc. in the calories per unit dry weight of the larvae were determined. The temperature changes from the microbomb calorimeter create a voltage profile consisting of a base fire, the voltage before the pellet is burned, followed by changes in voltage as the pellet burns (Fig. 4). The calories per unit dry weight of the larvae were obtained by determining the rise in temperature during combustion, correcting for changes in temperature before firing (the pre-fire slope) and after firing (the post-fire slope). The corrected rise was then converted to multiplying the adjusted rise (obtained by subtracting the corrected post-fire slope $([\text{Base fire} - 60\% \text{ of rise}] \times \Delta \text{Post-fire slope})$ from the corrected pre-fire slope $([\text{Base fire} - 60\% \text{ of rise}] \times \Delta \text{Pre-fire slope})$) by the calories, using the calibrated calories per rise for the benzoic acid standard. The benzoic acid pellet mass was multiplied by the benzoic acid combustion constant of 0.239 cal/J (6.318 cal mg⁻¹ / 26.435 J mg⁻¹) to convert joules to calories in the rise of benzoic acid. This was then divided by the adjusted rise from the benzoic acid to determine the calories per unit rise.

Calculations of growth in calories for the crab larvae followed the method described by Levine & Sulkin (1979). The change in the dry weight of each of the 2 groups of 15 larvae was found for Day 1 and Day 5 samples. The caloric content of the larvae, obtained from the bomb calorimeter measurements, was divided by the unit dry weight of the pellet. The calories per unit dry weight were then multiplied by the increase in dry weight between the newly-hatched larvae and 1-day or 5-day old larvae.

The measurements obtained from the total calories consumed and growth in calories were used to determine the gross growth efficiency and treatment comparisons were done to determine the difference in the gross growth efficiency between Day 1 control and high $p\text{CO}_2$ groups and between Day 5 and control and high CO_2 on each day.

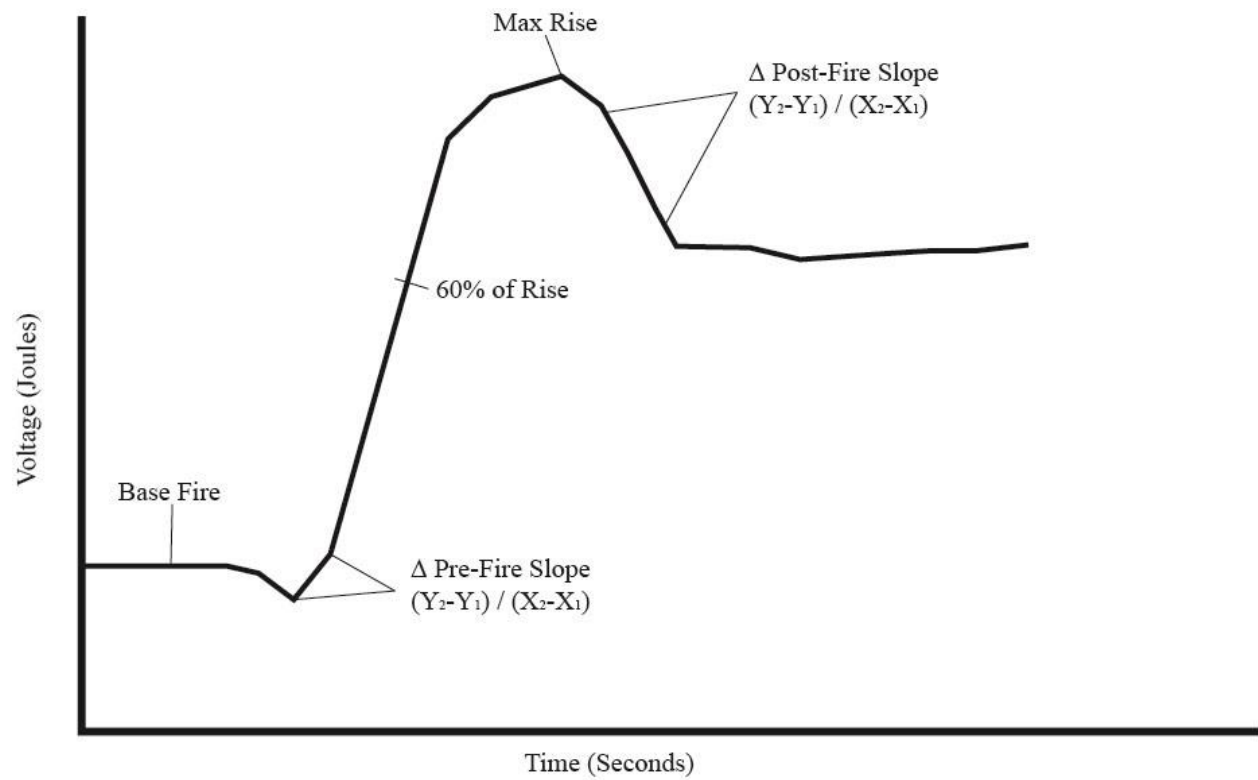


Figure 4: An example of the profile created by the WinDaq/XS software. This was used to determine the calories burned per unit dry weight for batches of 15 larval crabs.

Data Analysis

All statistical analyses were done using PASW 18 software. Data for all experiments (orthokinesis, klinokinesis, feeding rates and gross growth efficiency) were initially tested with two-way analysis of variance (ANOVA; $\alpha = 0.05$) with $p\text{CO}_2$ treatments and days of exposure as main effects. If the interaction term was significant, simple main effects contrasts were used to compare $p\text{CO}_2$ treatments on each day of exposure. The main effect contrasts are more powerful than a series of One-Way ANOVAs because they take both α and β errors into account.

The Levene's Test of Equality of Error Variances was used to test for equal variances prior to running the ANOVAs. If the assumption of equal variance was violated, the data were log transformed. If the log transformation failed to correct the violation, the α value was lowered to 0.025 to account for the violation (Gamst et al., 2008).

Results

Swimming Speed Studies

Orthokinesis

In Stage 1 *Metacarcinus magister*, mean swimming speed appeared higher for larvae exposed to high CO₂ than for those in control conditions on both days of exposure (Fig. 5). Additionally, larvae swam more on Day 1 than on Day 5. These observations were confirmed by the results of a two-way ANOVA (Table 2). Main effects of both treatment and days of exposure were significant. There was no significant interaction between CO₂ treatment and days of exposure.

Stage 1 *Hemigrapsus oregonensis*, showed a similar pattern of decreased swimming as the larvae aged (Fig. 6), but treatment effects were more equivocal. A two-way ANOVA again showed no significant interaction between treatment and days of exposure. Days of exposure significantly affected swimming with longer exposure time resulting in reduced swimming speed, but there was no significant difference in mean swimming speed due to treatment (Table 3).

To determine whether the larvae of the two crab species showed different responses to treatment conditions, they were compared for each day of exposure. For Day 1 of exposure (Fig. 7), a two-way ANOVA (Table 4) showed no significant interaction between treatments and species. However, there was a significant difference in swimming speeds of the two species and between the CO₂ treatments. *Metacarcinus magister* larvae had a higher swimming speed than *H. oregonensis* and the swimming speeds were higher in the high pCO₂ treatments than in the control conditions. By Day 5 there was no significant difference between the species or between

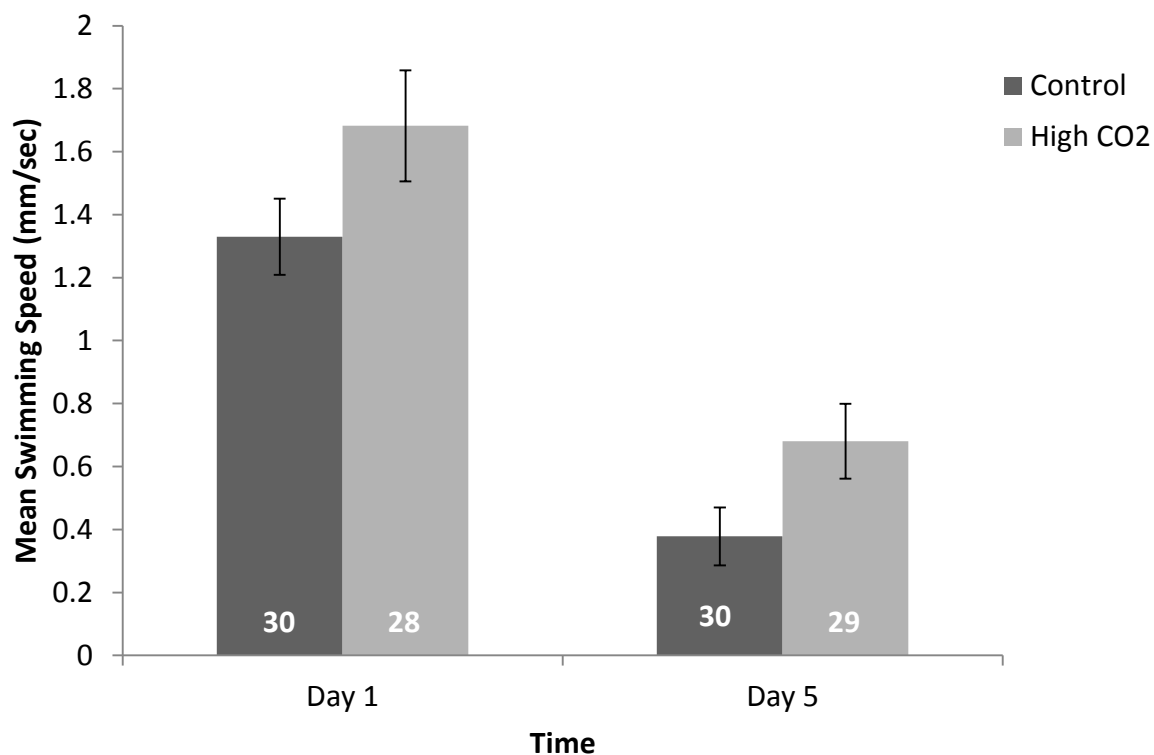


Figure 5: Mean swimming speed of larval *Metacarcinus magister* obtained by looking at the distance travelled per unit time in high (1000ppmv CO₂) and control (400ppmv CO₂) ocean acidification treatments. Numbers of replicates are indicated on the individual bars. Error bars indicate standard error values.

Table 2: Two-way ANOVA test comparing treatments (control and high CO₂) and days of exposure (one and five days post hatching) on *Metacarcinus magister* swimming speed ($\alpha = 0.05$).

Source	Sum of Squares	df	Mean Square	F	P
Treatments	3.12	1	3.12	6.06	0.01
Days	27.89	1	27.89	54.11	< 0.01
Treatment vs. Days	0.01	1	0.01	0.03	0.85

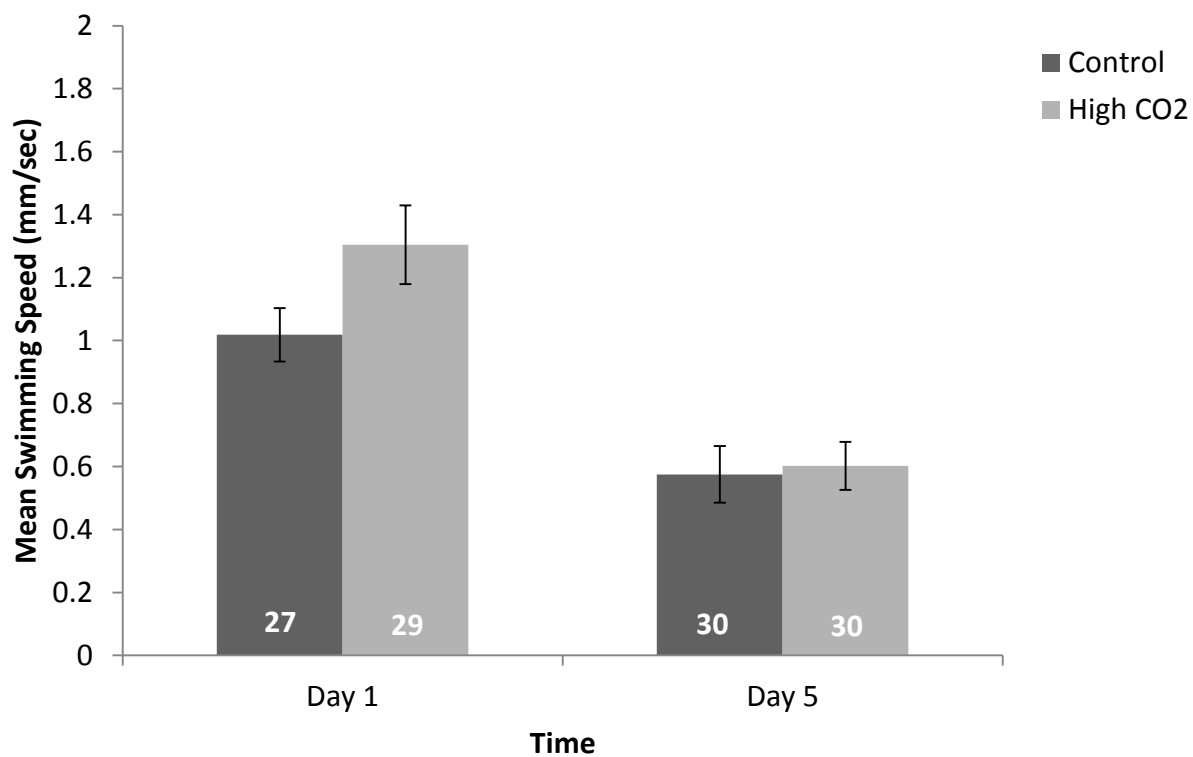


Figure 6: Mean swimming speed of larval *Hemigrapsus oregonensis* obtained by looking at the distance travelled per unit time in high (1000ppmv CO₂) and control (400ppmv CO₂) ocean acidification treatments. Numbers of replicates are indicated on the individual bars. Error bars indicate standard error values.

Table 3: Two-way ANOVA test comparing effects of treatments (control and high CO₂) and days of exposure (one and five) on *Hemigrapsus oregonensis* swimming speed ($\alpha = 0.05$).

Source	Sum of Squares	df	Mean Square	F	P
Treatments	0.70	1	0.70	2.54	0.11
Days	9.50	1	9.50	34.18	< 0.01
Treatment vs. Days	0.48	1	0.48	1.74	0.18

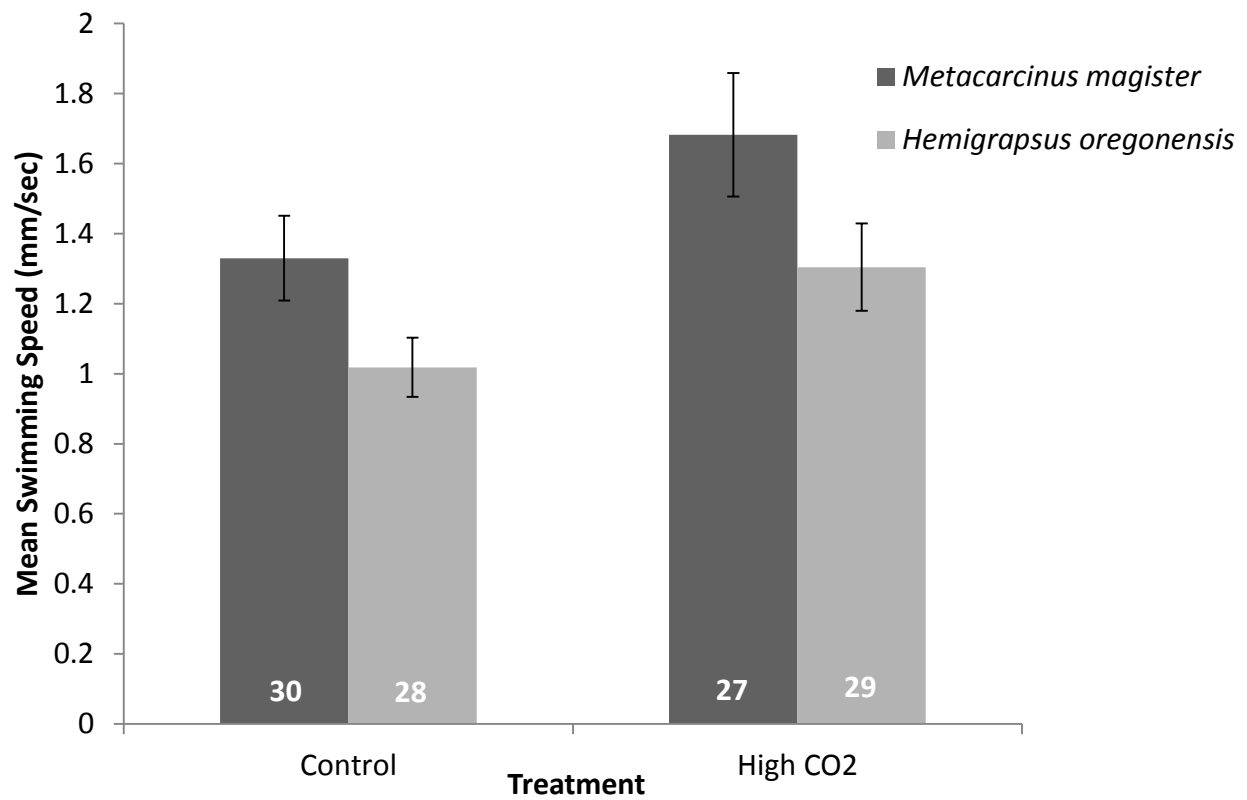


Figure 7: Day 1 mean swimming speed of larval *Metacarcinus magister* and *Hemigrapsus oregonensis* obtained by looking at the distance travelled per unit time in both the high (1000ppmv CO₂) and control (400ppmv CO₂) ocean acidification treatments. Numbers of replicates are indicated on the individual bars. Error bars indicate standard error values.

Table 4: Day 1 two-way ANOVA test comparing treatments (control and high CO₂) on *Metacarcinus magister* and *Hemigrapsus oregonensis* swimming speed ($\alpha = 0.05$).

Source	Sum of Squares	df	Mean Square	F	P
Treatments	2.89	1	2.89	5.48	0.02
Species	3.38	1	3.38	6.39	0.01
Treatment vs. Species	0.03	1	0.03	0.05	0.80

the treatments. The interaction was also not significant (Fig. 8, Table 5).

Klinokinesis

Mean numbers of turns while swimming are shown in Figure 9 for Stage 1 *Metacarcinus magister* larvae. Results suggest no difference due to the $p\text{CO}_2$ treatment for those exposed to the treatments for one day, with a decrease in mean number of turns after five days of exposure. The results of the two-way ANOVA showed significant interaction between treatment and days of exposure (Fig. 9, Table 6). Consequently, simple main effects contrasts were run (Table 7). Results showed a significant difference between treatments after five days of exposure but no difference after only one day (Table 7). There was also an overall decrease in the number of turns on Day 5 in both treatments.

Mean number of turns while swimming are shown in Figure 10 for stage 1 *Hemigrapsus oregonensis* larvae, suggesting no effect of treatment on turning. A two-way ANOVA showed no significant interaction between treatment and days of exposure, with main effects indicating no significant difference due to treatment (Table 8). Days of exposure, however, were significant, indicating a lower number of turns in older larvae.

When the number of turns taken per unit time was compared between the *M. magister* and *H. oregonensis* larvae after one day of exposure, larval *H. oregonensis* turned less often than did the larval *M. magister* in both the control and high treatments (Fig. 11). The results of the two-way ANOVA showed no significant interaction between treatment and species (Table 9), nor was there a significant treatment effect; however, there was a significant difference between the species ($p < 0.001$).

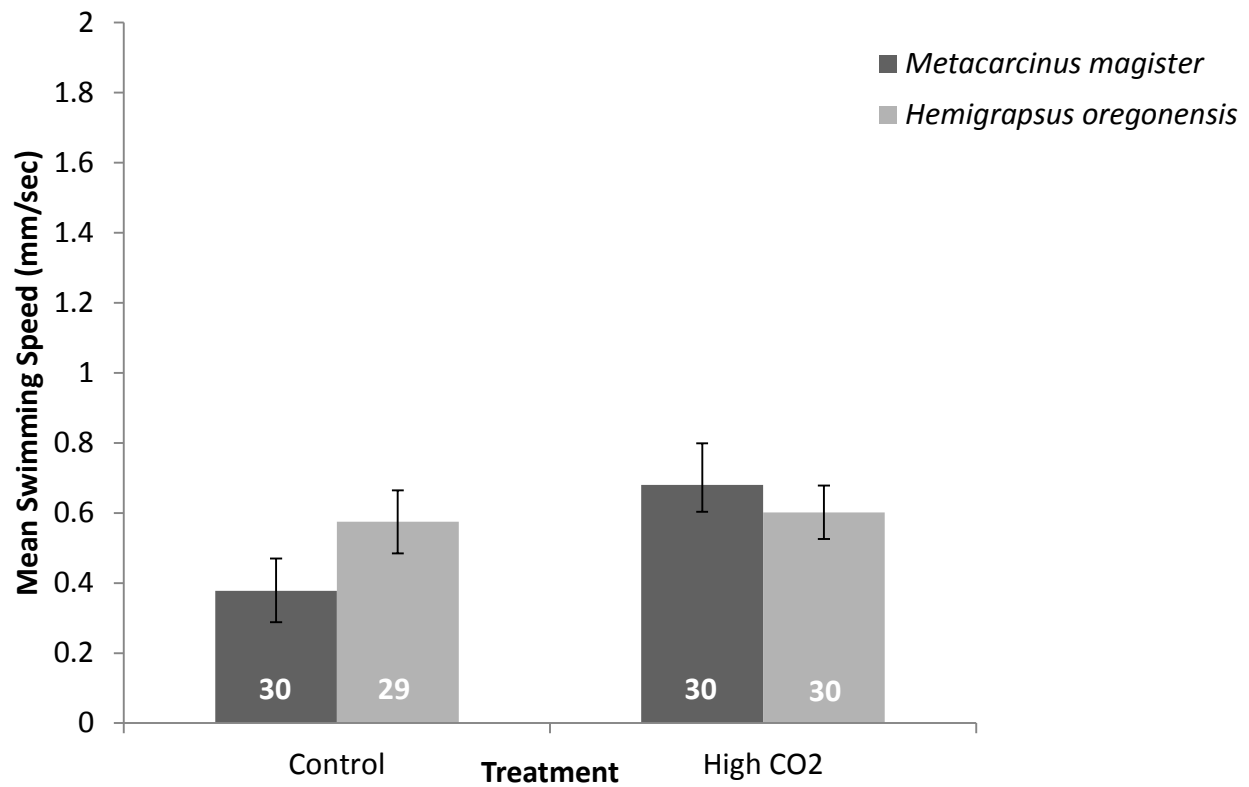


Figure 8: Day 5 mean swimming speed of larval *Metacarcinus magister* and *Hemigrapsus oregonensis* obtained by looking at the orthokinesis, the distance travelled per unit time in both the high (1000ppmv) and control (400ppmv) ocean acidification treatments. Error bars indicate standard error values.

Table 5: Day 5 two-way ANOVA test comparing treatments (control and high CO₂) on *Metacarcinus magister* and *Hemigrapsus oregonensis* swimming speed ($\alpha = 0.05$).

Source	Sum of Squares	df	Mean Square	F	P
Treatments	0.80	1	0.80	2.96	0.08
Species	0.10	1	0.10	0.38	0.53
Treatment vs. Species	0.56	1	0.56	2.07	0.15

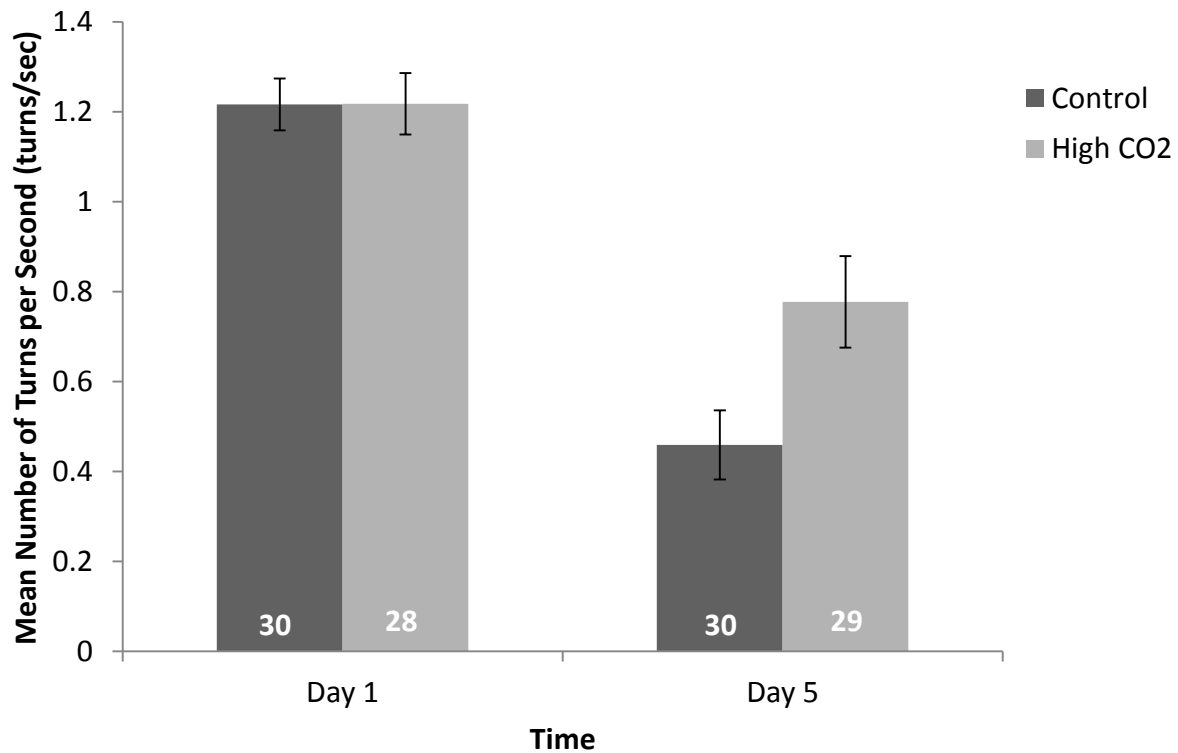


Figure 9: Mean number of turns per second of larval *Metacarcinus magister* obtained by looking at the number of turns taken per unit time in high (1000ppmv CO₂) and control (400ppmv CO₂) ocean acidification treatments. Numbers of replicates are indicated on the individual bars. Error bars indicate standard error values.

Table 6: Two-way ANOVA test comparing effects of treatments (control and high CO₂) and days of exposure (one and five) on *Metacarcinus magister* turning while swimming ($\alpha = 0.05$).

Source	Sum of Squares	df	Mean Square	F	P
Treatments	0.72	1	0.72	4.134	0.04
Days	10.18	1	10.18	58.092	< 0.01
Treatment vs. Days	0.71	1	0.71	4.056	0.04

Table 7: A series of simple main effects contrasts comparing effects of treatments (control and high CO₂) and days of exposure (one and five) on *Metacarcinus magister* turning while swimming ($\alpha = 0.05$).

Source	Sum of Squares	df	Mean Square	F	P
Day 1 control vs. high	4.61	1	4.61	25.82	0.58
Day 5 control vs. high	1.46	1	1.46	8.35	< 0.01

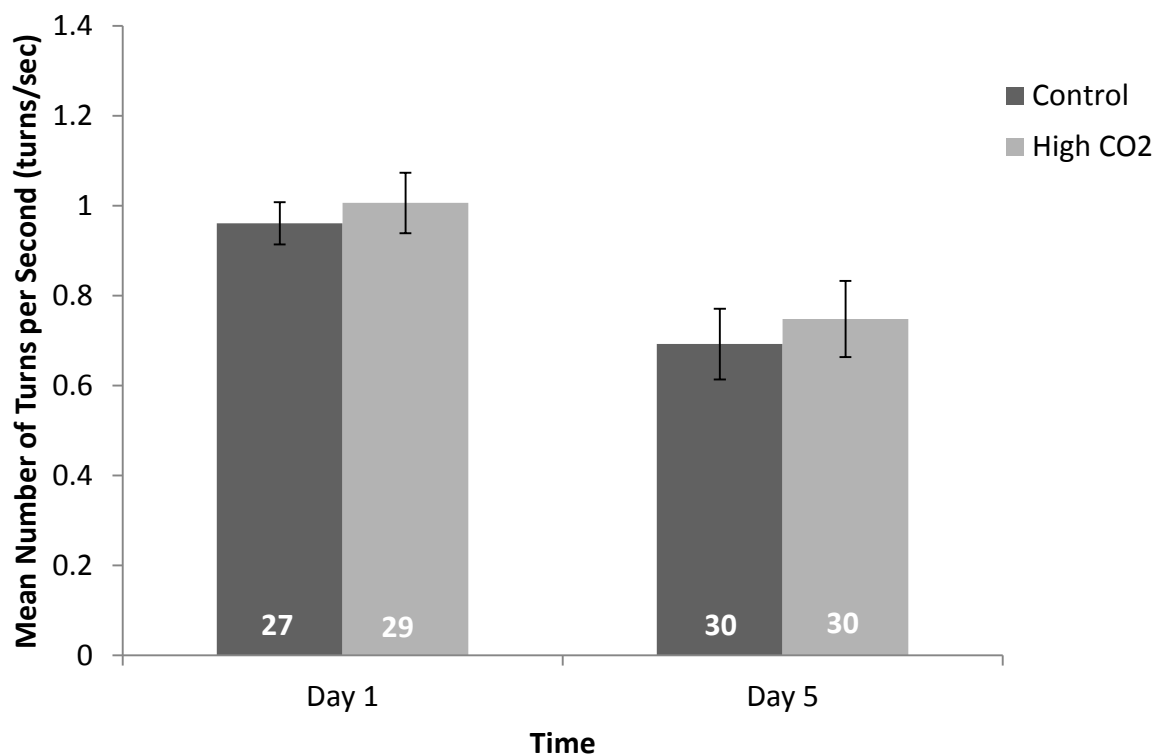


Figure 10: Mean number of turns per second of larval *Hemigrapsus oregonensis* obtained by looking at the number of turns taken per unit time in high (1000ppmv CO₂) and control (400ppmv CO₂) ocean acidification treatments. Numbers of replicates are indicated on the individual bars. Error bars indicate standard error values.

Table 8: Two-way ANOVA test comparing effects of treatments (control and high CO₂) and days of exposure (one and five) on *Hemigrapsus oregonensis* turning while swimming ($\alpha = 0.05$).

Source	Sum of Squares	df	Mean Square	F	P
Treatments	0.07	1	0.07	0.49	0.48
Days	2.02	1	2.02	13.31	< 0.01
Treatment vs. Days	0.001	1	0.001	0.005	0.94

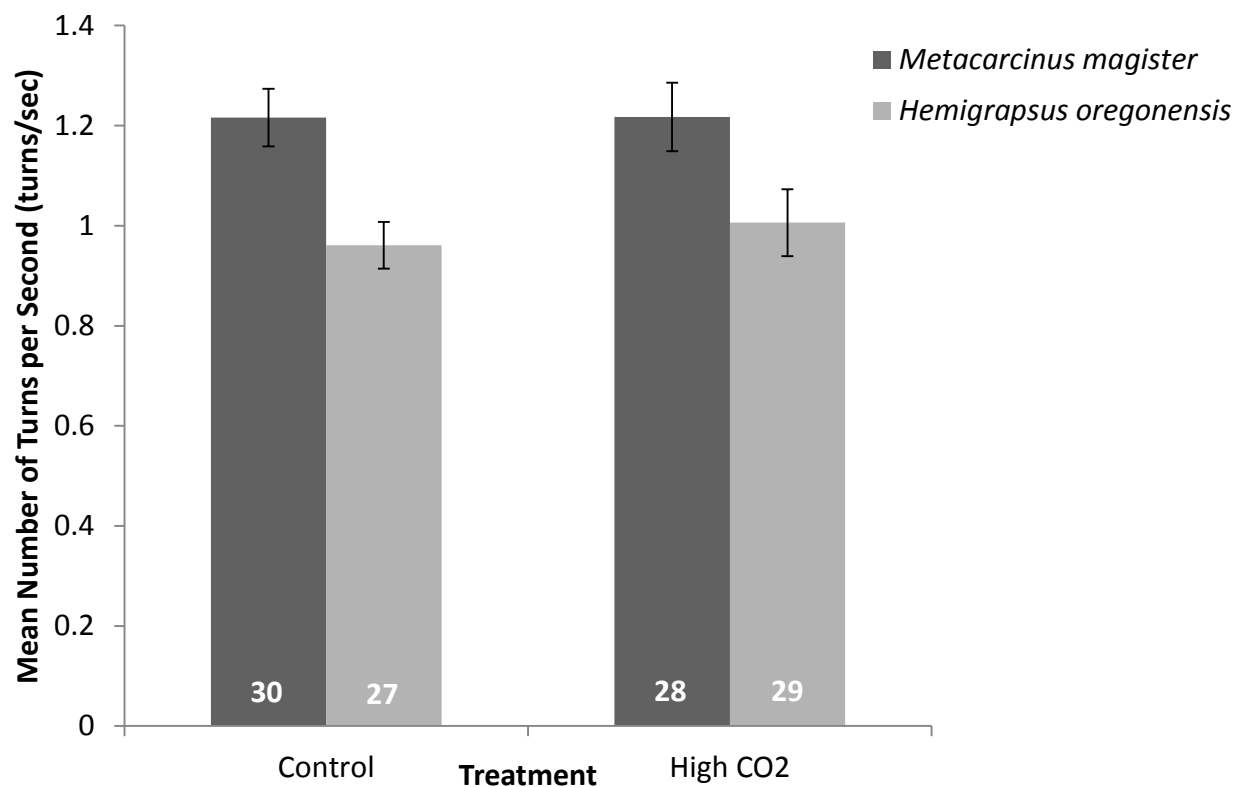


Figure 11: Day 1 mean number of turns in larval *Metacarcinus magister* and *Hemigrapsus oregonensis* in both the high (1000ppmv CO₂) and control (400ppmv CO₂) ocean acidification treatments. Numbers of replicates are indicated on the individual bars. Error bars indicate standard error values.

Table 9: Day 1 two-way ANOVA test comparing effects of treatments (control and high CO₂) in *Metacarcinus magister* and *Hemigrapsus oregonensis* turning while swimming ($\alpha = 0.05$).

Source	Sum of Squares	df	Mean Square	F	P
Treatments	0.01	1	0.01	0.13	0.71
Species	1.53	1	1.53	13.68	< 0.01
Treatment vs. Species	0.01	1	0.01	0.12	0.72

After one day of exposure, larval *M. magister* had a higher number of turns than did *H. oregonensis* (Fig. 11) but after five days, larval *M. magister* turned less often than did *H. oregonensis*, in the control conditions though there was no clear pattern in the high treatment (Fig. 12). Results of a two-way ANOVA indicated no significant interaction term. The effect due to species was not significant, but the effect from the treatment was significant (Table 10), indicating an increase in turning rate of the high treatment for both species after five days of exposure.

Feeding Rate Study

Feeding rate was determined by calculating the average number of *Artemia* sp. consumed in 24 hours by larval *Metacarcinus magister* and *Hemigrapsus oregonensis*. Larval *M. magister* mean feeding rate appeared higher for larvae exposed to control conditions than for those in high $p\text{CO}_2$ conditions for both days of exposure (Fig. 13). Five day old larvae consumed more *Artemia* sp. nauplii than did one day old individuals (Fig. 13). These observations were confirmed by a two-way ANOVA (Table 11) that showed no significant interaction between treatments and days of exposure but treatment and days of exposure were both significant.

Mean feeding rates shown in Figure 14 for stage 1 *H. oregonensis* larvae, suggest a possible difference in the feeding rate due the days of exposure, but not to treatment. A two-way ANOVA showed no significant interaction between treatment and days of exposure, and no significant difference due to treatment (Table 12). Days of exposure were significant, demonstrating an increase in the feeding rate of older larvae.

After one day of exposure, larval *H. oregonensis* consumed more *Artemia* sp. nauplii than did larval *M. magister* in both the control and high treatments (Fig.15). A two-way ANOVA

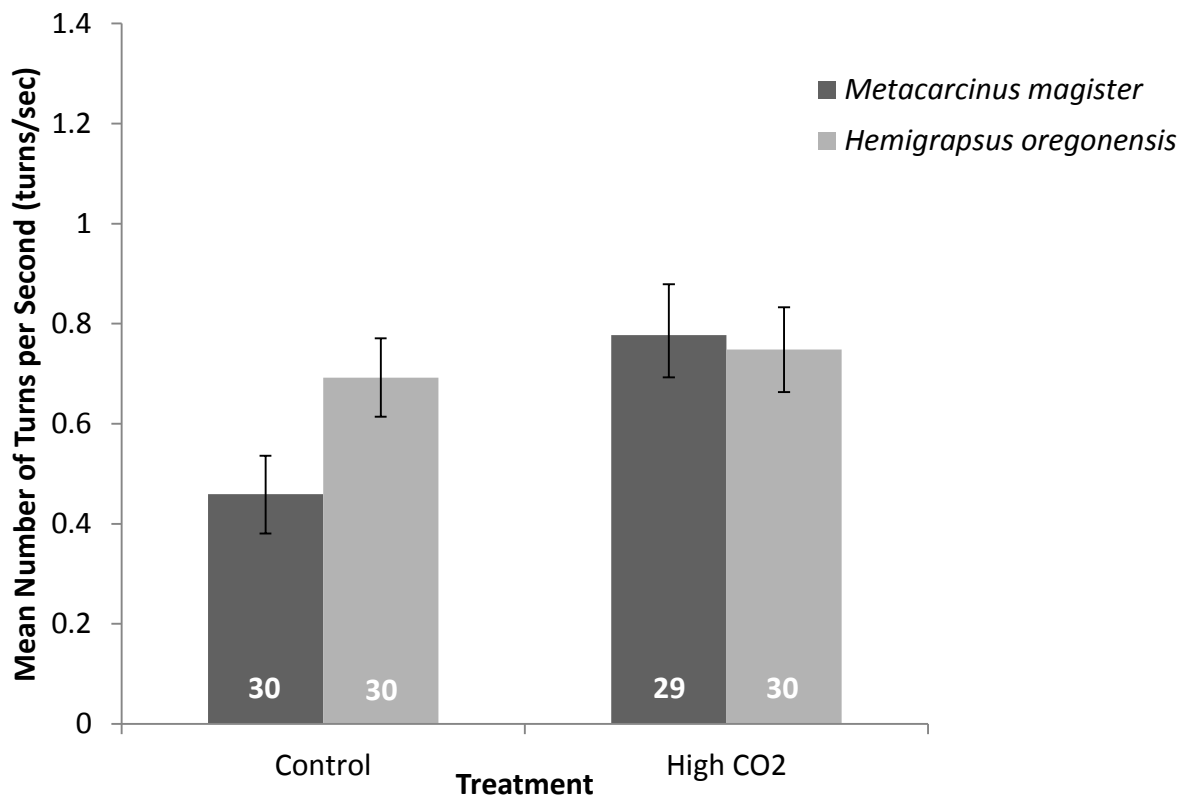


Figure 12: Day 5 mean number of turns in larval *Metacarcinus magister* and *Hemigrapsus oregonensis* in both the high (1000ppmv CO₂) and control (400ppmv CO₂) ocean acidification treatments. Numbers of replicates are indicated on the individual bars. Error bars indicate standard error values.

Table 10: Day 5 two-way ANOVA test comparing effects of treatments (control and high CO₂) in *Metacarcinus magister* and *Hemigrapsus oregonensis* turning while swimming ($\alpha = 0.025$).

Source	Sum of Squares	df	Mean Square	F	P
Treatments	1.02	1	1.02	4.84	0.03
Species	0.30	1	0.30	1.44	0.23
Treatment vs. Species	0.50	1	0.50	2.38	0.12

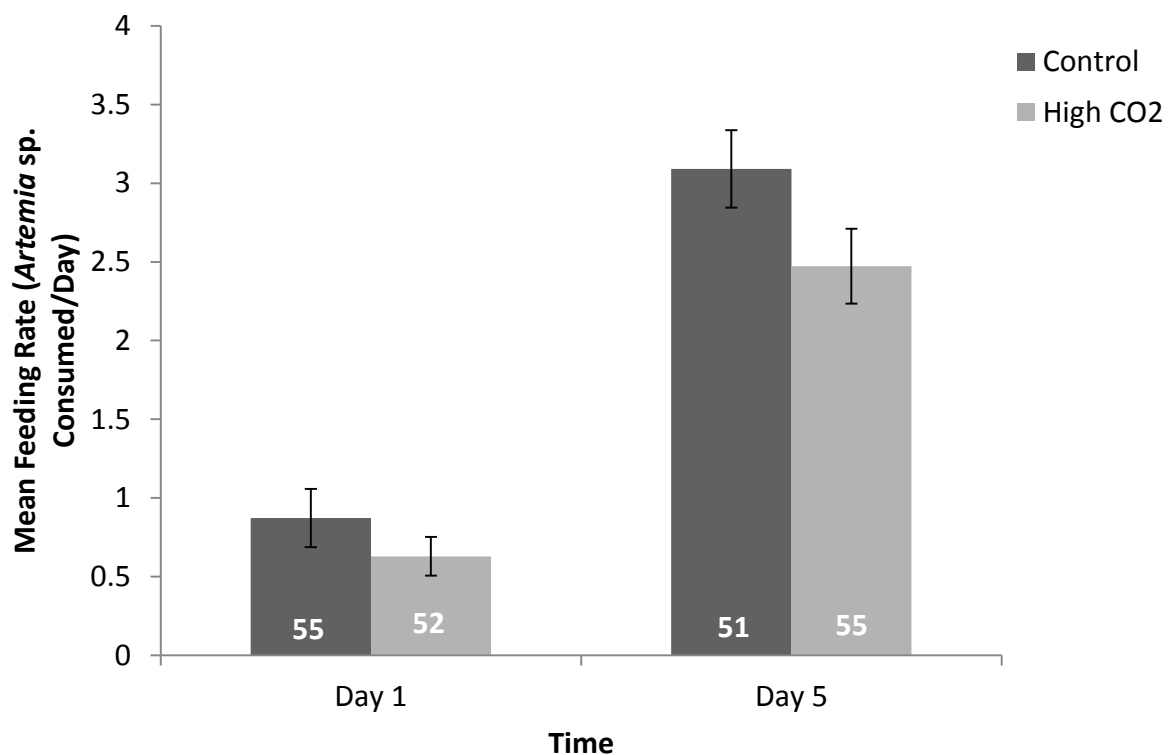


Figure 13: Mean feeding rate of larval *Metacarcinus magister* obtained by looking at the number of *Artemia* sp. consumed after one day and five days under high (1000ppmv CO₂) and control (400ppmv CO₂) ocean acidification treatments. Numbers of replicates are indicated on the individual bars. Error bars indicate standard error values.

Table 11: Two-way ANOVA test comparing effects of treatments (control and high CO₂) and days of exposure (one and five days post hatching) in *Metacarcinus magister* for feeding rate ($\alpha = 0.05$).

Source	Sum of Squares	df	Mean Square	F	P
Treatments	10.15	1	10.15	4.35	0.03
Days	225.74	1	225.74	96.88	< 0.01
Treatment vs. Days	1.92	1	1.92	0.82	0.36

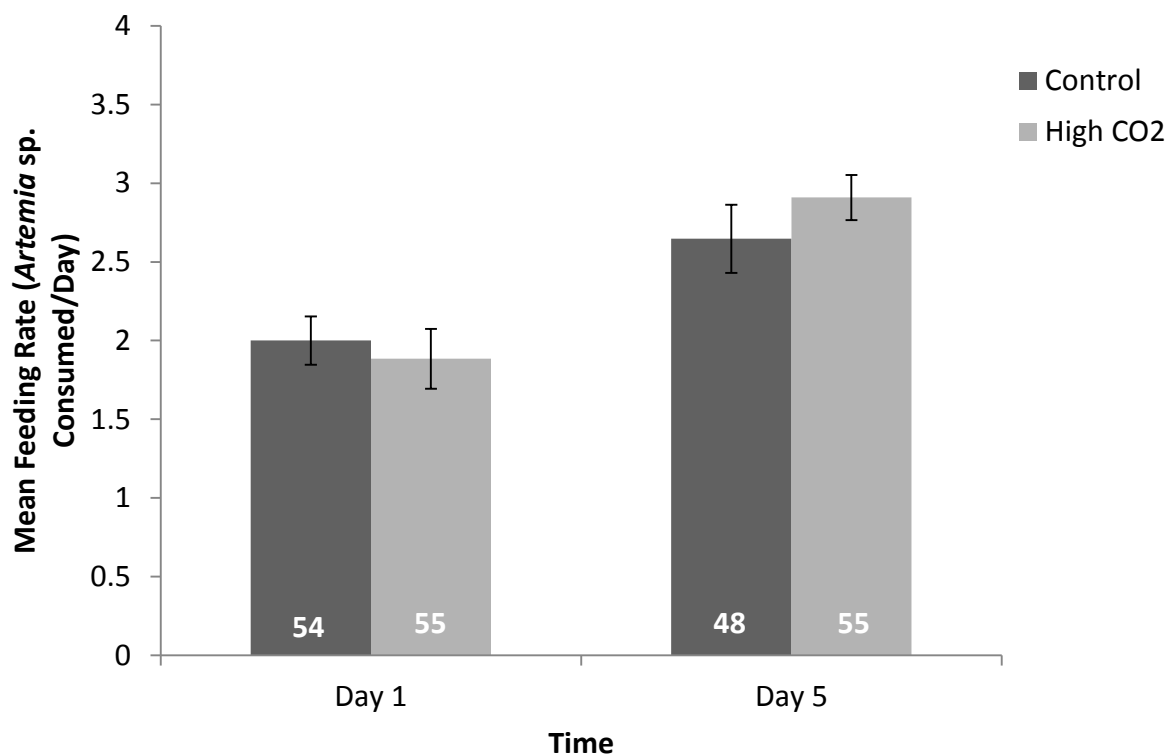


Figure 14: Mean feeding rate of larval *Hemigrapsus oregonensis* obtained by looking at the number of *Artemia* sp. consumed after one day and five days under high (1000ppmv CO₂) and control (400ppmv CO₂) ocean acidification treatments. Numbers of replicates are indicated on the individual bars. Error bars indicate standard error values.

Table 12: Two-way ANOVA test comparing effects of treatments (control and high CO₂) and days of exposure (one and five days post hatching) on *Hemigrapsus oregonensis* for feeding rate ($\alpha = 0.05$).

Source	Sum of Squares	df	Mean Square	F	P
Treatments	0.35	1	0.35	0.21	0.64
Days	36.20	1	36.20	21.35	< 0.01
Treatment vs. Days	1.71	1	1.71	1.01	0.31

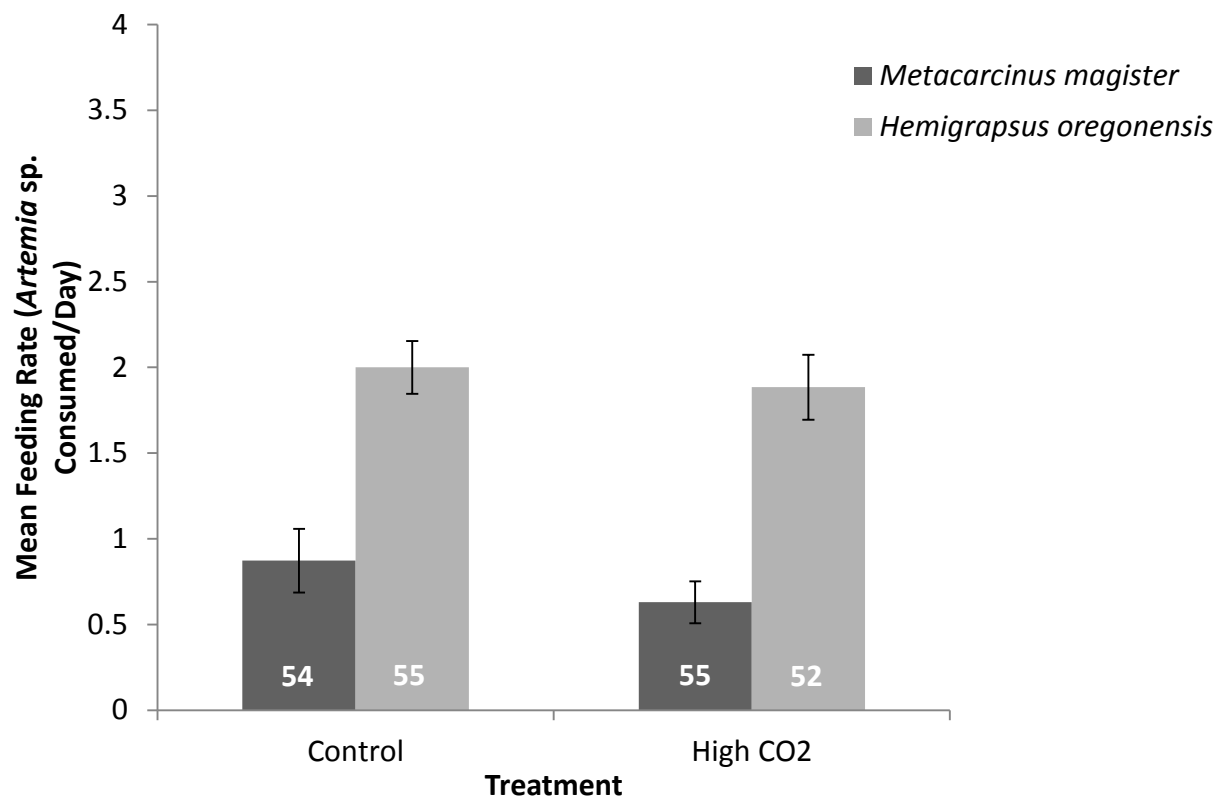


Figure 15: Day 1 mean feeding rate of larval *Metacarcinus magister* and *Hemigrapsus oregonensis* obtained by looking at the number of *Artemia* sp. consumed in both the high (1000ppmv CO₂) and control (400ppmv CO₂) ocean acidification treatments. Numbers of replicates are indicated on the individual bars. Error bars indicate standard error values.

showed the interaction between the two species was not significant (Table 13). The main effect for species was significant; however, the treatment effect was not (Table 13). On Day 5, the interaction between the species and treatment was significant (Table 14). Simple main effects contrasts showed a significant difference between the control and high CO₂ treatments for *M. magister* larvae but not for *H. oregonensis* (Fig. 16; Table 15).

Gross Growth Efficiency Study

Gross growth efficiency was obtained by estimating the proportion of calories the larvae consumed that was transformed into growth. After 24 hours, the *Metacarcinus magister* larvae exposed to control conditions appeared to have a higher mean gross growth efficiency than the high pCO₂ exposed larvae, but after five days of exposure, the reverse appeared true (Fig. 17). A two-way ANOVA showed no significant interaction between treatment and days of exposure (Table 16), and no significant difference between the main effects due to treatment or days of exposure effect. However, with only 2 replications per treatment, the power of the analysis was very low.

Table 13: Day 1 two-way ANOVA test comparing effects of treatments (control and high CO₂) in *Metacarcinus magister* and *Hemigrapsus oregonensis* for feeding rate ($\alpha = 0.05$).

Source	Sum of Squares	df	Mean Square	F	P
Treatments	1.56	1	1.56	1.03	0.30
Species	77.30	1	77.30	51.41	< 0.01
Treatment vs. Species	0.28	1	0.28	0.18	0.66

Table 14: Day 5 two-way ANOVA test comparing effects of treatments (control and high CO₂) on *Metacarcinus magister* and *Hemigrapsus oregonensis* for feeding rate ($\alpha = 0.05$).

Source	Sum of Squares	df	Mean Square	F	P
Treatments	1.71	1	1.71	0.67	0.41
Species	0.001	1	0.001	0.000	0.98
Treatment vs. Species	10.44	1	10.44	4.12	0.04

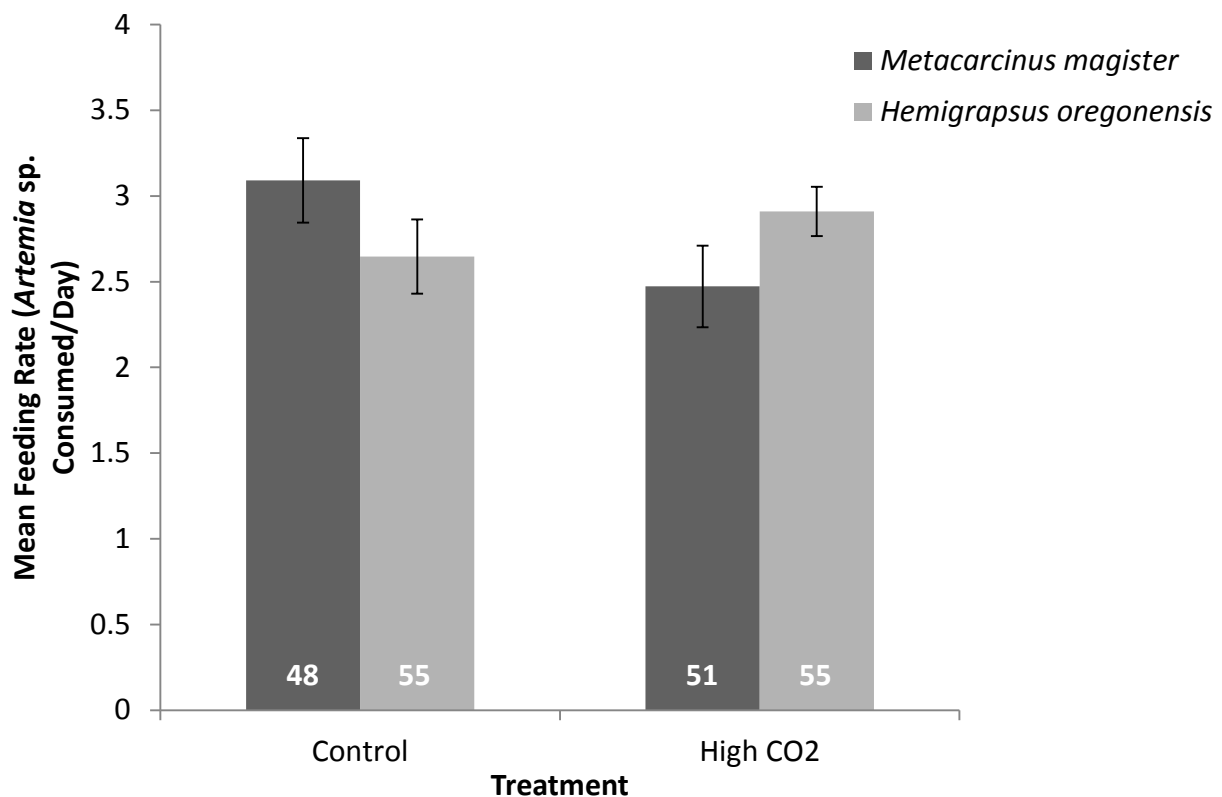


Figure 16: Day 5 mean feeding rate of larval *Metacarcinus magister* and *Hemigrapsus oregonensis* obtained by looking at the number of *Artemia* sp. consumed in both the high (1000ppmv CO₂) and control (400ppmv CO₂) ocean acidification treatments. Numbers of replicates are indicated on the individual bars. Error bars indicate standard error values.

Table 15: Day 5 simple main effects contrast for the interaction between the larval *Metacarcinus magister* and *Hemigrapsus oregonensis* for feeding rate ($\alpha = 0.05$), testing the effects of CO₂ treatment for each species.

Source	Sum of Squares	df	Mean Square	F	P
<i>M. magister</i>	10.50	1	10.50	4.15	0.04
<i>H. oregonensis</i>	1.81	1	1.81	0.18	0.39

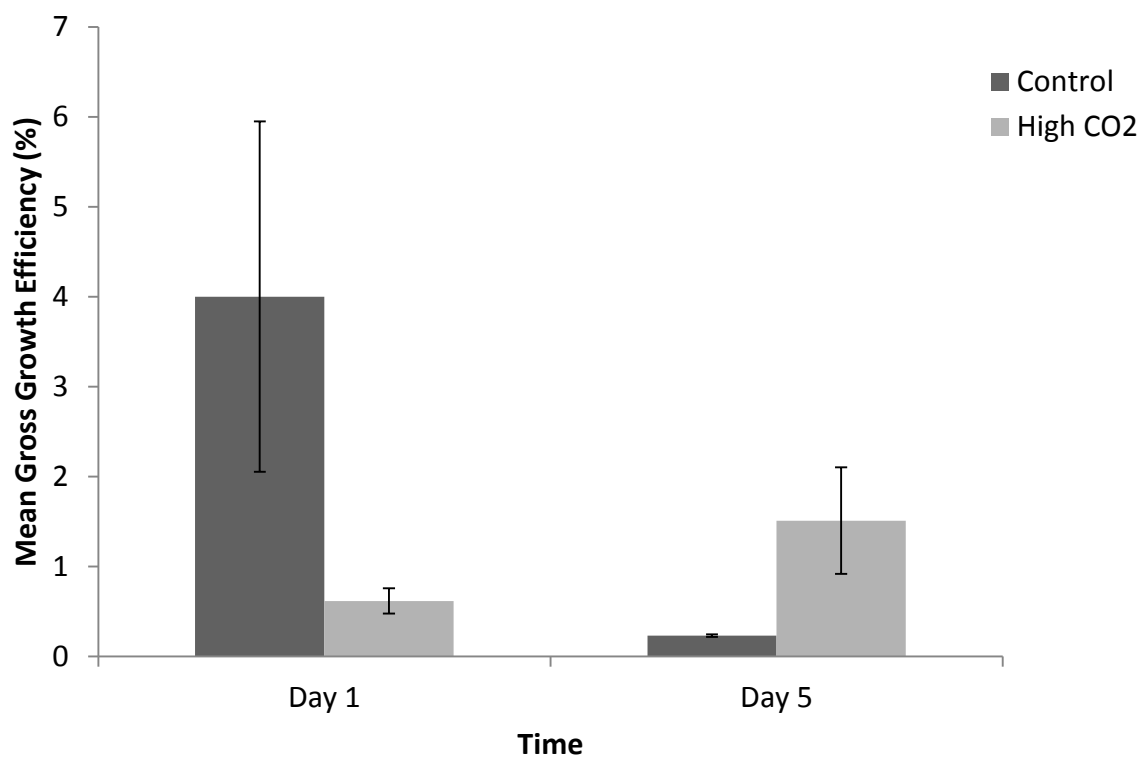


Figure 17: Mean gross growth efficiency of larval *Metacarcinus magister* obtained by looking at the percentage of growth in calories by the larvae over the calories they consumed after one and five days under high (1000ppmv CO₂) and control (400ppmv CO₂) ocean acidification treatments (n=2). Error bars indicate standard error values.

Table 16: Two-way ANOVA test comparing effects of treatments (control and high CO₂) and days of exposure (one and five days post hatching) on *Metacarcinus magister* for gross growth efficiency ($\alpha = 0.025$).

Source	Sum of Squares	df	Mean Square	F	P
Treatments	2.21	1	2.21	0.52	0.50
Days	4.13	1	4.13	0.98	0.37
Treatment vs. Days	10.86	1	10.86	2.59	0.18

DISCUSSION

Ocean acidification affects crab larvae in varying ways. When the duration of larval development was analyzed for *Metacarcinus magister* larvae under elevated CO₂ levels, there was no significant change found (Garcia, unpublished). An increase in the acidity of the seawater did not affect mortality in the larval stages of the intertidal porcelain crab, *Petrolisthes cinctipes* (Ceballos-Osuna et al., 2013). Though there was a slight increase in total lipids, larval *P. cinctipes* maintained their protein content and rate of metabolism when kept in either the low (574±105 µatm) or high pCO₂ (1361±199 µatm) treatments (Carter et al., 2013). However, when the cold-eurythermal larvae of the spider crab *Hyas araneus* were placed in elevated CO₂ conditions, their lipid content, C: N ratio, and growth rate all decreased. As a result, there was an increase in the time spent in each larval stage (Walther et al., 2010). After seven days of exposure to seawater containing a high level of pCO₂ (962.5 µatm), larvae from the red king crab *Paralithodes camtschaticus* increased their calcium content and size (Long et al., 2013). These results indicate that any effect on larval development due to ocean acidification will likely vary among crab species. Little is known about the effects of ocean acidification on larval crab behavior.

Vertical Distribution of Larvae

Changes in vertical distribution of planktonic organisms can have a significant effect on their dispersal. In the case of larval forms, including larval crabs, vertical distribution can have direct implications for geographic distribution of settling populations and indirect effects on access to prey and encounters with unfavorable environmental conditions. Sulkin (1984) proposed a model for vertical distribution and depth regulation in larval crabs that is composed of three basic elements: buoyancy, primary orientation and swimming activity.

Buoyancy is a manifestation of the physical properties of the organism. Because the density of a larval crab exceeds the density of the volume of water it displaces, it exhibits negative buoyancy; that is, if totally inactive, it will sink. The rate of sinking will be governed by density, shape, and other morphological features (e.g., spines or length of appendages) and can be measured by rendering the larva unconscious. The orientation of the larva during such an inactive phase (its “default orientation” according to Sulkin, 1984) is governed by a variety of largely morphological factors (e.g., the larva’s center of gravity). Thus, if a larval crab is inactive, it will rotate and sink with its dorsal side is down. Downward movement, therefore, requires only inactivity on the part of the larva.

This default condition can be altered by taxis and kinesis responses. A taxis response to environmental stimuli causes the larva to adjust its primary orientation in space. Such response may simply complement the default passive orientation, resulting in downward movement by an active larva or it can oppose the default passive orientation, resulting in upward movement when the larva begins to move by swimming. Typical taxis stimuli in the marine environment include gravity (geotaxis) and light (phototaxis). Light, however, is a complicated stimulus that can induce both taxis and kinesis responses. Its effects are dependent on wavelength and intensity, and its threshold can be influenced by the larva’s previous exposure history (Sulkin, 1984). Because of the variability and unpredictability of light as a taxis stimulus, Sulkin (1984) suggested that the very conservative geotaxis response might provide better cues and well serve as the primary cue for a larva’s vertical movement behavior. This response could be further modified by light or other cues that are directional in the marine environment in the vertical plane. Most larval crabs, especially in their early stages, show negative geotaxis, a response where response to gravity will position them to move upward when they swim.

How much and how fast larval crabs swim will determine their actual movement in the vertical plane and is itself subject to environmental cues. Such responses are referred to as “kinesis.” Kinesis is not directional and has been shown to be subject to a wider range of cues, including light (see above for complicating factors), salinity, temperature, and pressure (Sulkin, 1984). Because of its ubiquitous nature, its total predictability, and its change with depth, hydrostatic pressure resulting in barokinesis is of particular significance. Typically, planktonic organisms, including larval crabs, show high barokinesis; that is, they swim faster as pressure increases, an outcome that will occur as they move deeper (or are hatched at depth). This behavior in response to hydrostatic pressure is shown by larvae of the deep sea red crab, *Geryon quinquedens*. Kelly et al. (1982) found that swimming rates for *G. quinquedens* increased with an increase in pressure of 10 and 20 atm. In the absence of light, Stage 1 zoea of the crab *Leptodius floridanus* showed similar increased swimming speeds with an increase in the hydrostatic pressure (Sulkin, 1973). Bentley and Sulkin (1977) observed the larval swimming responses of the xanthid crab *Rhithropanopeus harrisii* under different levels of hydrostatic pressure, and found that all zoeal stages increased mean swimming speed as hydrostatic pressure increased. Tankersley et al. (1995) similarly found that megalopae from the blue crab, *Callinectes sapidus*, and the fiddler crab, *Uca* spp., migrated upward with an increase in hydrostatic pressure.

The combination of these three factors (buoyancy, primary orientation and activity level), and the ways in which they interact during the course of larval development, influences the patterns of vertical movement and distribution of larval crabs in the water column. A model suggested by Sulkin (1984) proposed a negative feedback system in which larval crabs, which typically swim in bursts interspersed with periods of inactivity, will sink as they stop swimming.

Their vertical orientation then will cause them to swim upwards when increased pressure triggers their power stroke. Thus swimming will occur for longer periods or more frequently as they sink into deeper water and are exposed to increasing pressure. The result is an upwards movement. As they ascend the water column, they will be exposed to decreasing pressure that will result in less frequent and/or slower swimming that will eventually result in their resuming the sinking mode that carries them back downward. Thus they can maintain their relative position in the water column, with precision with their response governed in part by the sensitivity of their kinesis response. As environmental and developmental features change on time scales from seconds to weeks, the interactions among the regulating factors will likely change resulting in changing vertical distribution patterns. This could result, for example, in diurnal vertical migration and/or changes in vertical distribution during ontogeny. Of course, there may be many factors complicating this simple model, for example light, salinity, and temperature. Forward (1974) studied the light and dark adaptations of Stage 1 larvae of the estuarine mud crab, *Rhithropanopeus harrisi* (Gould), at different light intensities. Results showed that, after light adaption, swimming speed during negative geotaxis (swimming against gravity) was significantly higher than during positive geotaxis (swimming with gravity) at the highest light intensities. Tankersley et al. (1995) found that megalopae of the blue crab, *Callinectes sapidus*, and the fiddler crab, *Uca* spp. migrated upward more frequently with an increase in salinity. When larval blue crabs, *Callinectes sapidus*, were exposed to decreased temperatures, there was a decrease in the overall swimming speed in stages four and seven larvae (Sulkin et al. 1980). The model predicts that activity level of the larvae will be a very significant component of the regulation of depth and access to prey that may be distributed differentially with depth. These patterns of distribution can by extension affect growth, survival, and ultimately dispersal.

Swimming Speeds and Ocean Acidification

In the present study, when compared to that of the larvae placed in the control treatment, exposure to increased ocean acidification resulted in an increase in larval swimming speeds in *Metacarcinus magister* larvae (Fig. 5; Table 2), although swimming speeds in both control and high ocean acidification exposure declined after five days. In *Hemigrapsus oregonensis* larvae, however, there was no comparable effect on the swimming speed. Two conclusions can be drawn from these results. Firstly, response to acidification conditions is species-specific in larval crabs. In *M. magister*, this response may be accentuated under conditions typical of ocean acidification exposure. Secondly, in newly-hatched larvae of both species, initial swimming activity is comparatively high, then declines significantly after five days. High swimming speeds may be adaptive in newly-hatched larvae for crab species that hatch on the bottom and must quickly move up in the water column to encounter suitable prey.

This overall decrease in the swimming activity with time in both species could be the result of the larva's preparation for molting. Energy available for locomotion could be conserved as molting approaches. Stage one molting in larval *M. magister* occurred seven to 10 days after hatching when the larvae kept in a laboratory environment (Garcia et al., 2011). Wolcott and Hines (1990) found that the blue crab, *Callinectes sapidus* began to decrease its swimming speed three to five days before molting occurred.

The effect of the greater number of turns taken per unit time in *Metacarcinus magister* (Fig 9; Table 6) on Day 5 of exposure to ocean acidification on vertical migration may depend upon how this response interacts with other traits such as primary orientation and orthokinesis response. Undirected movement created by frequent turning could affect vertical distribution by

lengthening the time it would take larvae to move through the water column. Metaxas (2001) found that crabs with a greater swimming speed had a higher dispersal rate and wider dispersal area.

In *M. magister*, increased swimming speeds under acidified conditions may result in tighter control of the larval vertical positioning in the water column as they are subject to increasing and decreasing hydrostatic pressure more rapidly. This will not be the case with *H. oregonensis* larvae. If maintaining a characteristic depth is important in their life histories, changes in swimming behavior could create higher sinking rates in *M. magister* due to their greater mass compared to *H. oregonensis*. Changes in larval swimming speed due to the increase in ocean acidification could have an effect on larval vertical distribution. Increases in swimming speeds could help larvae move faster through the water column allowing them to capture prey more effectively.

Feeding and Predator – Prey Interactions

In this study, CO₂ enrichment had no significant impact on feeding rate of *Metacarcinus magister* (Fig. 13; Table 11) or *Hemigrapsus oregonensis* larvae (Fig. 14; Table 12). Larval crabs are considered encounter feeders, meaning they try to ingest anything they contact (Baylon et al., 2004). Changes in vertical distribution caused by changes in larval swimming speed could affect encounters with prey. Because most crab species release larvae on the bottom, the larvae must move vertically up into the water column to encounter denser patches of prey. Indeed, most larvae possess traits that will move them, upon hatching; quickly to the photic zone where the variety of prey, including small zooplankton they need to develop normally, are located (Sulkin, 1984). In species such as *M. magister* in which acidification conditions may accelerate such

upward movement, larvae could benefit from the response, getting the newly hatched larvae to an area with a higher chance to encounter prey. Of course, this assumes that the ocean acidification condition do not fundamentally change the distribution behavior of the prey. Intertidal species such as *H. oregonensis* may not be as sensitive to the need to move significant vertical distances to improve prey encounter rates as they do not cover a large prey capture area.

Changes in larval turning rates created by exposure to decreases in ocean pH could have indirect effects on both larval feeding and predation. In terms of turning behavior, such behavior may indeed increase the likelihood of encounters with prey. The increase in the number of turns seen in *M. magister* with ocean acidification exposure could therefore prove beneficial. In their natural environment, a crab larva's diet consists of a variety of other zooplankton including copepods. Studies done on the effects of changes in the ocean's acidity have found no significant change in copepod egg production or abundance (Niehoff et al., 2013; Weydmann et al., 2012) but it is unclear what role the condition of the prey played in the crab larvae's feeding ability. A future approach to this feeding rate study could involve hatching *Artemia* sp. nauplii under the corresponding CO₂ conditions before feeding them to the crab larvae. Feeding rates might have differed if the *Artemia* sp. nauplii faced environmental changes similar to those experienced by the crab larvae. There is no information on how change in pH could affect *Artemia* sp. nauplii's behavior.

As far as avoiding predation, increased swimming speeds and/or turning behavior could have either beneficial or harmful effects. The outcome will likely depend upon the predator, its size and behavior. An increase in the erratic movement by the larval crabs could make them more accessible to visual predators or make them harder to capture. Assessing this potential impact on larval success will require specific experiments with specific predators, an approach

beyond the scope of the present study. Constant movement also creates constant movement of the surrounding water. This could attract predators that rely on pressure fronts caused by water displacement to find their prey (Laudek, 1983). This interaction is difficult to predict as we have little evidence on how increased levels of CO₂ might affect the organisms that feed on crab larvae.

Growth Efficiency and Ocean Acidification

In the present experiments, gross growth efficiency measured the effectiveness of *Metacarcinus magister* larva in transferring the calories they consumed into energy stored in their tissues during growth. Though the control larvae on Day 1 appeared to be more efficient in transferring their calories to growth (Fig. 17) than were the high CO₂ conditioned larvae on Day 5, there was no statistical evidence to show that an increase of ocean acidification affected gross growth efficiency (Table 16). Though there is a pattern, the small sample size reduces the confidence in the statistical outcome. Levine and Sulkin (1979) found the gross growth efficiency of Stage 1 *Rhithropanopeus harrisi* larvae to be 10.6%. Growth efficiency was 11.84% for Stage 1 *Menippe mercenaria* (Mootz & Epifanio, 1974). The values obtained in this experiment were lower than those obtained in these studies. This could be due to the difference in diet between the experiments. Both Levine and Sulkin (1979) and Mootz and Epifanio (1974) used *Artemia* sp. nauplii to measure growth efficiency while this experiment used rotifers. The lack of significance in the gross growth efficiency experiment could also be due to the short duration of the experiment. Extending the duration of this experiment through the first molt in this and other studies could give a better understanding the effects of ocean acidification over time. Also, it is difficult to obtain significant caloric values when measuring larval crabs as the

bomb calorimeter measures the calories burned from organic matter. Crab larvae are comprised of organic matter and a large proportion of calcareous exoskeleton which does not burn.

Intertidal vs. Subtidal Spawning Crab Species

The *Metacarcinus magister* larvae had an overall higher swimming speed than *Hemigrapsus oregonensis* larvae on Day 1 (Table 4, 5). *M. magister* larvae also had a higher turning rate on Day 1 (Table 9) and the difference was even greater on Day 5 (Table 10). This could be due to the difference in habitat of the two species. *H. oregonensis* are found in a constantly changing intertidal environment, with constantly changing tides and exposure to rainfall. Water inputs from river sources can reduce the alkalinity of shallow intertidal habitats (Salisbury et al. 2008). Wootton et al. (2008) found that the intertidal pH level can change by 1.5 units annually due to varying weather conditions increasing the freshwater input and daily tidal changes. Located in this constantly changing environment, *H. oregonensis* larvae may have developed adaptations to avoid behavioral changes even under fluctuating seawater chemistry. These adaptations could explain why there was no change in the swimming speeds of these larvae under elevated CO₂.

Future of Ocean Acidification Studies on Larval Crabs

Several improvements could be made to these experiments to help us to better understand the effects of ocean acidification on larval crab swimming speeds, feeding rates, and gross growth efficiency. It would be interesting to lengthen the experimental time through molting to zoeal stage two. This would present an observation of the effects of ocean acidification over a complete molting cycle. It would also be interesting to analyze the effects of ocean acidification on the swimming speeds of other crab species like *Cancer oregonensis* which would provide

another winter spawning and smaller size crab and *Hemigrapsus nudus* which would provide another summer spawning crab. It may be interesting to study the effect increased ocean acidification on the larvae when placed in acidified conditions from the embryonic stage, taking initial larval development under consideration.

Crustaceans play a significant role in the world's economy and the ocean's food web. But as the ocean's acidity increases, the effects on crustaceans still remain unclear. Few papers have been published on the effects of ocean acidification on larval crab behavior and how these changes could affect larval distribution. With these looming changes in the ocean chemistry, the need for research analyzing its effect on calcifying organisms is increasingly important. Results from this research show that after days of constant exposure to high levels of $p\text{CO}_2$, larvae decreased their overall activity level in *Metacarcinus magister*. However, after five days, there was an increase in the erratic movement in *M. magister* larvae placed in the high treatment when compared to those placed in the control treatment. There was also no evidence that changes in ocean acidification directly affected larval feeding rates or gross growth efficiency for larvae of either crab species. However, changes in larval turning rates and swimming speeds could have an indirect effect on feeding rates and gross growth efficiency. These results suggest that ocean acidification could affect larval dispersal and the role of the crab larvae in the food web. This data indicates the importance of continuing this research on the larval stage of other commercially important animals like the American lobster, *Homarus americanus*, and the Olympic oyster, *Ostrea lurida*.

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